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THE PROVENTRICULUS OF *CICINDELA SEXGUTTATA* FABRICIUS (COLEOPTERA : CICINDELIDAE)[1]

BY W. W. JUDD[2]

Abstract

The proventriculus of *Cicindela sexguttata* Fabricius is described. It is similar in form to that of the proventriculi of Adephaga described by authors. It is oval in shape, and its inner lining is organized into eight longitudinal folds bearing a coating of fine hairs.

Materials and Methods

Specimens of *Cicindela sexguttata* Fabricius were collected on gravel roads about London, Ont. during September, 1938. They were identified with a key to the genus *Cicindela* in Blatchley (4).

The digestive system was removed from a specimen, under water, with the aid of a binocular microscope and was immediately placed in Bouin's fixative. After being fixed for about 10 hr. the tissue was cleared of fixative by repeated changes of 70% alcohol and was stored in 70% alcohol. For histological study the digestive tract was sectioned (10μ) with a rotary the sections were stained with haemotoxylin and eosin.

Description

The proventriculus of *Cicindela sexguttata* is oval and long (P, Fig. 4). Its anterior end is embedded in the posterior end of the voluminous crop (C, Figs. 1, 4); its posterior end is narrowed and enters the anterior end of the mid-gut (MG, Fig. 4). The mid-gut crosses the abdominal cavity at right angles to the length of the proventriculus and its outer surface bears numerous finger-like caeca.

The body of the proventriculus is roughly rectangular in transverse section (P, Fig. 1). Its innermost lining is a thin sclerotized intima bearing a coating of fine hairs (CH, Fig. 2). Beneath the intima is the epithelial layer (EP). The whole lining of the proventriculus is organized in the form of eight longitudinal folds. Four of these are narrow partitions (CP), which, in transverse section, appear as finger-like processes projecting into the lumen of the proventriculus. Alternating with these are four ridges (CR) each

2.

CH
LM
CR
CP
EP
CM
C

0.19 mm.

1.

P
C

0.7 mm.

3.

CR
CP
CM
I
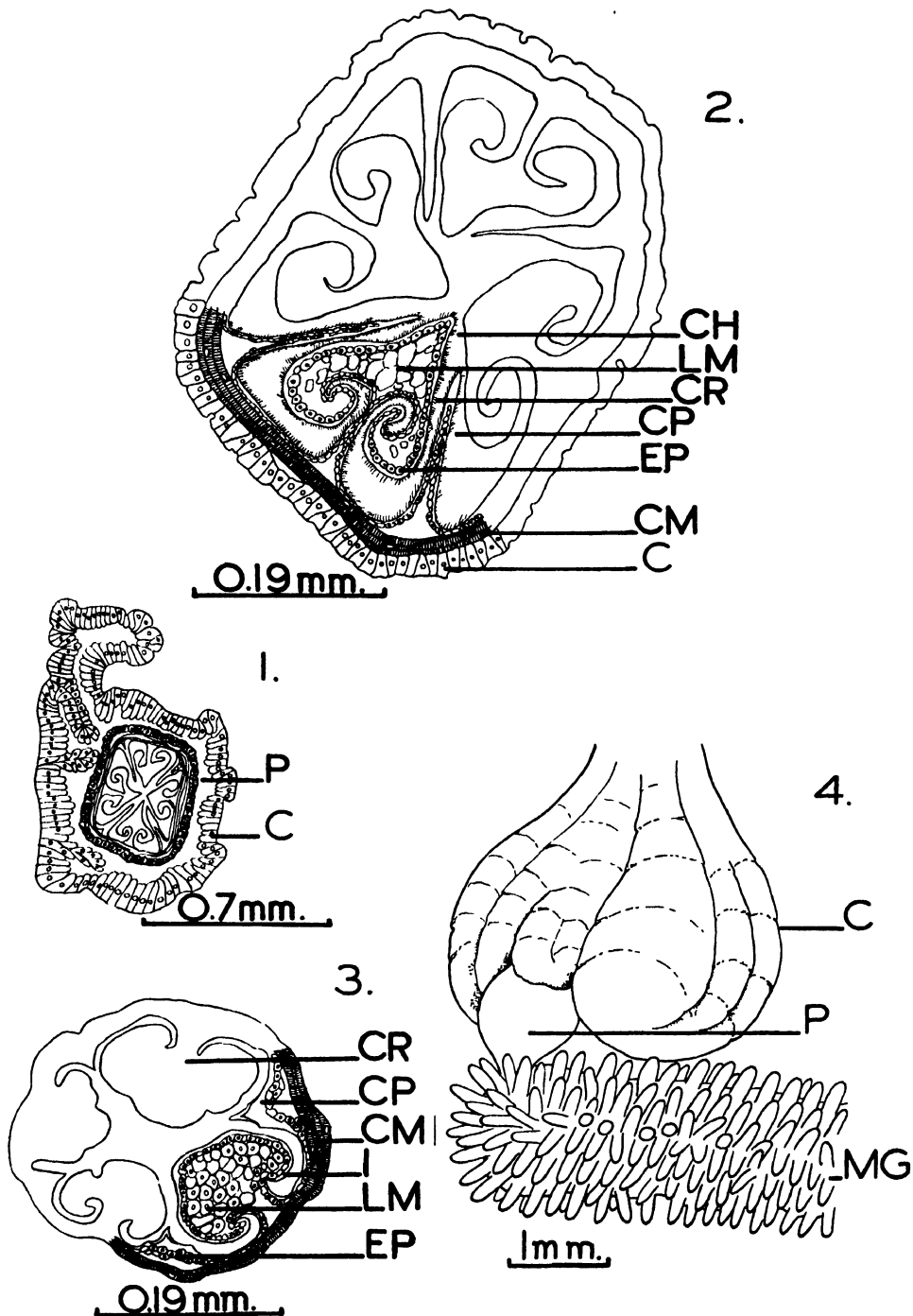LM
EP

0.19 mm.

4.

C
P
MG

1 mm.

FIG. 1. *Transverse section of body of proventriculus and crop.* FIG. 2. *Transverse section of body of proventriculus.* FIG. 3. *Transverse section of posterior end of proventriculus.* FIG. 4. *Part of digestive tract showing crop, proventriculus, and mid-gut.*

C: crop; CH: coating of hairs; CM: circular muscle; CP: partition of proventriculus; CR: ridge of proventriculus; EP: epithelium; I: intima of proventriculus; LM: longitudinal muscle; MG: mid-gut; P: proventriculus.

attached to the wall of the proventriculus by a very narrow base. In transverse section these ridges are roughly triangular, one corner of the triangle projecting into the lumen, and the other two curled inward toward the base. The cavities of these ridges contain strands of longitudinal muscle (LM). The outer covering of the proventriculus is circular muscle (CM), three to four strands thick. Closely applied to the outer surface of the proventriculus is the epithelium of the crop.

The narrow posterior region of the proventriculus is roughly circular in transverse section (Fig. 3). The partitions (CP) are considerably wider in this region than in the body of the proventriculus, while the four ridges (CR) are rounded rather than triangular. The sclerotized intima (I) does not bear a coating of hairs.

Discussion

The foregoing description indicates that the structure of the proventriculus of *Cicindela sexguttata* is similar to that of the proventriculi of other beetles of the Adephaga described by authors. Descriptions of the proventriculus of four species of Carabidae were made by Bess (3), Judd (6), Schaefer (7), and Whittington (9). In these the proventriculus has eight longitudinal folds, as in *C. sexguttata*, but of these the four ridges are rounded rather than triangular in transverse section. In his description of the Dytiscidae Bordas (5) shows that this organ has eight teeth, four large and four small. Balfour-Browne (1, 2) also describes four "main" and four "intermediate" lobes in the proventriculus of the Dytiscidae. Thiel (8) concludes that in the Adephaga the proventriculus is a well developed structure with strong longitudinal and circular muscle.

Acknowledgment

The writer wishes to express his gratitude to Prof. J. D. Detwiler, University of Western Ontario, for his interest and guidance during the course of this work.

References

1. BALFOUR-BROWNE, F. The proventriculus in the Dytiscidae as a taxonomic character. Stylops, London, 3 : 241-244. 1934.
2. BALFOUR-BROWNE, F. The proventriculus in the Dytiscidae as a taxonomic character. Second Note. Stylops, London, 4 : 191. 1935.
3. BESS, H. A. Alimentary canal of *Calasoma sycophanta* Linnaeus. Ohio J. Sci. 35 : 54-64. 1935.
4. BLATCHLEY, W. S. The coleoptera or beetles of Indiana. Nature Pub. Co., Indianapolis. 1910.
5. BORDAS, L. Le gésier des Dytiscides. Compt. rend 156 : 1703-1705. 1913.
6. JUDD, W. W. The proventriculus of a ground beetle, *Chlaenius* sp. (Coleoptera, Carabidae). Can. Entomologist, 79 : 58-60. 1947.
7. SCHAEFER, P. E. The alimentary canal of *Sphaeroderus nitidicollis* Chev. var. *schaumi* Chd. (Coleoptera). Ohio J. Sci. 31 : 406-415. 1931.
8. THIEL, H. Vergleichende Untersuchungen an den Vormägen von Käfern. Z. wiss. Zool. 147 : 395-432. 1936.
9. WHITTINGTON, F. B. The alimentary canal of *Harpalus pennsylvanicus* Dej. Ohio J. Sci. 35 : 131-138. 1935.

CHITWOODIELLA OVOFILAMENTA GEN. ET SP. NOV., A NEMATODE PARASITE OF GRYLLOTALPA[1]

By M. A. Basir[2]

Abstract

Chitwoodiella ovofilamenta gen. et sp. nov. is described from *Gryllotalpa*. This new genus can be differentiated from other genera of the subfamily Thelastomatinae, by its long, striated buccal cavity, its characteristic filament-bearing eggs, and the position of the origin of the ovaries, both of which originate in the anterior region of the body from about the middle of the oesophagus.

In a previous paper (3) the writer has described five new nematode worms from mole crickets of the genus *Gryllotalpa*. During the continued study of the parasites of this group of insects, a new species, with an unusual method of egg production, was found. It belongs to the subfamily Thelastomatinae of the family Thelastomatidae (4, 5). It has a different structure from other representatives of this subfamily and cannot be accommodated in any known genus (1, 2, 6). Accordingly, the writer regards it as representing a new genus and a new species of the subfamily Thelastomatinae and the name *Chitwoodiella ovofilamenta* is proposed for it.

Description

Genus Chitwoodiella gen. nov.

Generic diagnosis: Thelastomatinae.

Male unknown.

Female with mouth opening circular, surrounded by a circumoral elevation and eight simple papillae. Amphids appear as small circular openings. Buccal cavity characteristic, very long and annulated. Oesophagus also very long, about one-fourth of the length of the body, consisting of a long corpus followed by a narrow isthmus and a posterior valvular bulb. Tail attenuated filiform. Vulva between middle and posterior third of body. Vagina short and directed anteriorly. Two ovaries, both anterior, originating in the oesophageal region a little behind the nerve ring. Uteri divergent. Eggs elliptical, organically connected with each other and enveloped by filamentous threads that arise in the form of a tuft from each pole; segmented before deposition and laid in the form of a chain.

Type species: Chitwoodiella ovofilamenta sp. nov.

Specific diagnosis: Chitwoodiella.

Male unknown.

Female 1.11 to 2.15 mm. long by 200μ in maximum width. Striations present only in the cervical region; striae 10 in number. First annule 10μ wide, remaining annules 5.3μ wide. Mouth circular, surrounded by a

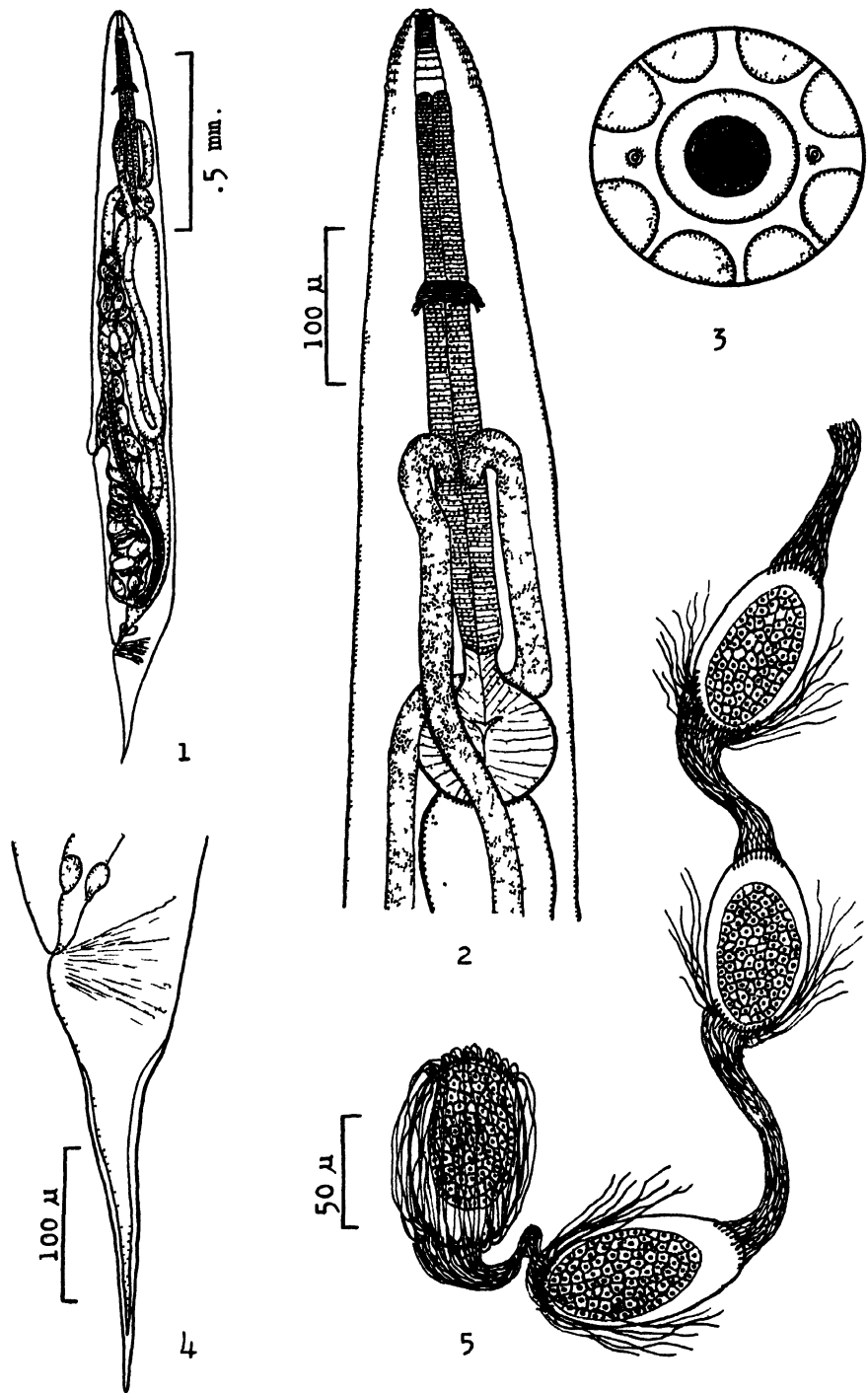FIG. 1. *Female, entire, lateral view*   FIG 2 *Female, head, lateral view*
FIG 3 *Female, en face view*   FIG 4 *Female, tail, lateral view*
FIG. 5. *A portion of the egg chain as laid by female (slightly pressed under the cover slip).*

circumoral elevation and eight simple papillae. Amphids present. Buccal cavity 53μ long, striated throughout its length, its anterior half being very finely striated and narrower in width than its posterior half; in the latter the striations are more distinct, wider apart, and fewer in number. Minimum width of the buccal cavity 10μ, maximum width, up to 18μ. Oesophagus 300 to 475μ long, consisting of a corpus 255 to 370μ long by 30μ at its maximum width, an isthmus 22 to 25μ long by 20μ wide and a posterior valvular bulb 60 to 80μ long by 60 to 85μ wide. Nerve ring 105 to 180μ from the anterior end of the body. Intestine enlarged anteriorly to form a cardia. Anus 150 to 290μ from the posterior end of body. Tail attenuated filiform. Vulva salient, projecting a little outwards, 1.25 mm. from the anterior end of body. Vagina short, directed anteriorly. Ovaries two, both anterior, originating in the oesophageal region a little behind the nerve ring. Uteri divergent. Eggs elliptical, 80μ long by 40μ wide, segmented before deposition, laid in the form of a chain being connected with each other and enveloped by threads that arise from each pole.

Host: *Gryllotalpa africana*.

Location: Intestine (rectum).

Type locality: Aligarh (North India).

Discussion

Egg filaments have been observed in a variety of nematodes belonging to different groups. They have been reported in species belonging to *Mermis*, *Tetrameres*, *Rhabdocona*, *Cystidicola*, *Metabronema* (7), *Binema* (10), and *Pseudonymous* (8, 9). However, in the family Thelastomatidae egg filaments have been recorded on two occasions only; one in *Pseudonymous* where a single filament arises and winds itself round the egg, and the other in *Binema* where filaments arise as polar tufts, and the filaments of two or more eggs form a loose network or shell, in which the eggs are lodged. They are laid in two or rarely in threes or even fours. In the present worm no such enclosure or secondary shell is formed; the eggs are laid singly, being connected with each other in the form of a chain. One peculiar thing about these eggs is that they are not separate but appear to be organically connected with each other by the filaments One end of each of these filaments arises from one egg, and the other end is connected to the next egg. At the proximal pole of the egg, which passes out of the vulva last, the implantation of the filaments is light and only threads of the connecting tuft are attached. On the other pole, the implantation is thick and heavy, because, in addition to the attachment of the filaments of the tuft itself, numerous other filaments arise from this pole, curving round and enveloping the egg on all sides. These remain attached to the egg surface by some adhesive substance. This becomes clear on pressing the eggs, when the threads separate from the sides as shown in the figure. The connecting filaments are developed somewhere in the oviduct.

This raises a question that cannot be answered without further study, namely the method by which the eggs are laid. It seems probable that once egg laying starts, it cannot finish until the whole of one uterus is emptied

completely. Moreover, it seems probable that all the eggs from one uterus must pass out before the eggs from the other uterus can start to pass out. If this is not the case, the only alternative seems to be the breaking of the chain. However, the tuft of filaments is so heavy and stout that this seems to be improbable. When the eggs were pressed too heavily on a slide under a cover slip, the egg shells broke down but the connecting threads were still unbroken: This makes the possibility of their breaking up during ·normal egg laying even more improbable.

Apart from the slight resemblance in the presence of polar filaments on the eggs, this genus has little in common with the genera *Binema* and *Pseudonymous*. However, it shows some resemblance to the genus *Cephalobellus* Cobb, 1920, in the form of its body, but differs from it in the following points. In *Cephalobellus* the buccal cavity is very short, while in *Chitwoodiella* it is long and striated. In the latter, both ovaries arise in the oesophageal region, and the eggs bear polar filaments; in the former, one of the ovaries arises anteriorly and the other posteriorly, and the eggs have no polar filaments. The proportionate lengths of the oesophagus and tail are different in these two genera; in the former, both the oesophagus and the tail are comparatively much shorter than in the latter.

Because of the differences in the form of its long and striated buccal cavity, its characteristic filament-bearing eggs, and the position of the origin of the ovaries, this genus can be differentiated from all other genera of the subfamily Thelastomatinae. The writer believes that these differences are sufficient to justify the erection of a new genus.

References

1. BASIR, M. A. Nematodes parasitic in Indian-cockroaches. Proc. Indian Acad. Sci. B, 12 : 8-16. 1940.
2. BASIR, M. A. Two new nematodes from an aquatic beetle. Proc. Indian Acad. Sci. B, 13 : 163-167. 1941.
3. BASIR, M. A. Nematodes parasitic in *Gryllotalpa*. Rec. Indian Museum, 44 : 95-106. 1942.
4. CHITWOOD, B. G. A synopsis of nematodes parasitic in insects of the family Blattidae Z. Parasitenk. 5 : 14-50. 1932.
5. CHITWOOD, B. G. and CHITWOOD, M. B. Nematodes parasitic in Philippine cockroaches. Philippine J. Sci. 52 : 381-393. 1933.
6. CHRISTIE, J. R. Some nemic parasites (Oxyuridae) of Coleopterous larvae. J. Agr. Research, 42 : 463-482. 1931.
7. CHRISTIE, J. R. An introduction to nematology. Sec. 2, Part 2, M. B. Chitwood, Babylon, N. Y.
8. GALEB, O. Recherches sur les entozoaires des insectes. Organisation et développement des oxyuridés. Arch. zool. exptl. gén. 7 : 283-390. 1878.
9. TODD, A. C. A new parasitic nematode from a water scavanger beetle. Trans. Am. Microscop. Soc. 61 : 286-289. 1942.
10. TRAVASSOS, L. Quelques nématodes du *Gryllotalpa*. Compt. rend. soc. biol. 93 : 140-141. 1925.

STUDIES ON THE HOUSEFLY (*MUSCA DOMESTICA* L.)

I. THE BIOLOGY AND LARGE SCALE PRODUCTION OF LABORATORY POPULATIONS[1]

By A. Wilkes,[2] G. E. Bucher,[2] J. W. MacB. Cameron,[3] and A. S. West, Jr.[4]

Abstract

Using the Peet-Grady method for propagating houseflies required as biological test animals, variations were observed that had considerable bearing on production and subsequent adult life. Differences in length of life, fecundity, time of emergence of the adults from puparia, and the onset of egg laying occurred between different populations of flies. Investigations were carried out to determine the extent and causes of the variations and to develop more suitable techniques for producing large numbers on a more accurately predictable basis. Variability in production of puparia was found to be due largely to the age of female stock and rate of fermentation in the rearing medium through their effects on egg hatchability and larval survival and excessive crowding caused by high temperatures. By using eggs from genetically-selected stock of known age and rearing in a temperature-controlled medium, production of flies was increased and maintained at a constantly uniform rate. A description is given of the equipment and methods used.

Introduction

The housefly, *Musca domestica* L., is often used as a biological means of determining the effectiveness of insecticides in the establishment of control measures for many insect pests. In 1932 the Peet-Grady Method was adopted as an official test, having been designed to provide a standard means of evaluating insecticidal sprays in association with an Official Test Insecticide as a basis for comparison. In 1938 the Large Group Modification of the Peet-Grady Method was officially adopted in which larger groups of flies are used as test units. Both methods are being used extensively at the present time. An outline of the methods is given in Blue Book, Soap and Sanitary Chemicals, 1943 (1).

As biological tests the Peet-Grady methods are subject to the variations normally found among any group of living animals. It is necessary, therefore, that all tests should be carried out under comparably standard conditions in order that reasonable agreement can be obtained in the evaluation of the results. To do this, definite practices are followed involving the use of representative units of flies reared and tested in a prescribed manner and under certain uniformly standard conditions. In the production and handling of the flies certain routine methods of procedure are always followed particularly with regard to the rearing medium, incubation of the larvae, separation

of the pupae, and handling of the adults. Even under these conditions, however, the results are not always as uniform as might be desired. This was found to be particularly so in the present work.

During the current series of investigations large quantities of flies were required for test purposes. Throughout the studies, it was essential that a fixed and often large number of them should be available for testing on a predetermined date and that biological variations between the different populations were as small as possible. Using the Peet-Grady rearing methods a number of difficulties were encountered and consequently, studies were undertaken to determine the causes and the possibility of developing more suitable techniques for producing the flies. The purpose of the present paper is to present the results obtained in this phase of the work with the hope that they may be of value to others engaged in similar biological assays.

The General Method of Rearing

The method first used for the production of flies followed closely the standard Peet-Grady procedure. A stock of flies was obtained by placing 500 puparia of the 'Powell' strain in a standard fly rearing cage 12 in. × 12 in. × 12 in. in a well-illuminated room maintained constantly at 27° C. and 50% relative humidity. After emergence the adults were provided daily with a mixture of equal parts of water and milk in small stender dishes, two to each cage, in which were placed pieces of paper towelling. Eggs were deposited by the females either on the towelling or on the moist edges of the dishes. The eggs were removed each morning and agitated in water before being separated into lots for breeding by volumetric estimations from a calibrated pipette.

The medium used for rearing the larvae is a modification of Richardson's formula (Richardson (2)) and consisted of the following ingredients:

Soft wheat bran —four battery jars (approximately 15,000 cc.)
Alfalfa meal —two battery jars (approximately 7500 cc.)
Malt extract —50 cc.
Bakers' yeast cake—94 gm.
Water —three and two-thirds battery jars (approximately 13,600 cc.)

This mixture was placed in cylindrical battery jars measuring 6 in. in diameter by 9 in. high to a depth of within 2 in. of the top.

In each battery jar 2500 eggs were stirred lightly beneath the surface of the medium and the jar covered with a damp cloth. After three days the cloth was allowed to remain dry. On the morning of the ninth day of incubation, pupation of the larvae was almost complete, the puparia usually being formed in the dry upper layer of the medium The top inch of medium was then removed, spread on trays and placed in front of a fan until it was sufficiently dry to effect a clean separation of the pupae from the medium by

inclining the tray in the direct path of the air stream. Usually the puparia from the different jars were mixed and the number determined by weight. Incubation of the puparia was carried out at 26.7° C.

Variations in Puparial Production

During the propagation of flies using the method indicated, considerable variation in production was encountered. The yield of puparia varied not only from one battery jar to another but also between different generations. This was equally true of the relative sizes of the puparia.

At a density of feeding larvae represented by the number hatching from 2500 eggs per battery jar the mean weight of the puparia was 19.5 gm. per thousand, the standard deviation being 1.5 gm. For every increase of 500 eggs per jar the weight per one thousand puparia decreased by approximately 0.8 gm. Thus, size was an index of crowding of the larvae during rearing and could be changed by unfavorable conditions in the medium. In many cases abnormally small puparia were produced when the temperature of the rearing medium became too high. In these jars the larvae crowded into the layer of medium in which the temperature was lower i.e. nearer their preferred temperatures, and were to be found in masses, usually around the outer walls of the jars.

On the basis of the number of puparia reared from battery jars 'seeded' with 2500 eggs comparisons could readily be made. Although, normally, after eight days of incubation pupation of the larvae in the jars was complete, approximately 2% of the larvae usually failed to pupate within the eight day period. By discarding the culture after eight days of incubation the loss of these larvae was not particularly noticeable, since adult emergence from the puparia they formed was abnormally low. Usually the presence of these late maturing larvae indicated unsatisfactory rearing conditions such as excessive crowding, lack of sufficient food, or low temperatures and they constituted less than 0.1% of the puparia reared.

In determining the number of puparia reared per jar, estimates were made on the basis of the total weight of puparia and the weight of at least two counted samples of 500. The error of estimate was found by experiments to be 0.5%. For purposes of comparison production was expressed as the number of puparia per battery jar unit of rearing medium either as whole numbers to the nearest 10 or as the mean weight per jar-unit of puparia.

The most striking variable encountered during the production of flies was in the number of puparia per unit-jar of rearing medium. This is illustrated in records taken from one generation of laboratory-reared stock.

Lot No. 3, mean total production per jar = 39.3 gm.
   No. 7, mean total production per jar = 21.2 gm.
   No. 7, Jar *a*, total production per jar = 29.4 gm.
   No. 7, Jar *b*, total production per jar = 15.7 gm.
   No. 7, Jar *c*, total production per jar = 18.5 gm.

The average weight per puparium was 0.0195 gm. This represents a maximum difference in the production of over 60%. From a study of the differences a number of factors were found to be involved, an outline of which is given under the headings indicated.

The Age of Parental Females

It was clearly evident that as the age of the ovipositing females increased the number of puparia reared per jar decreased. This is shown in Table I. In the table is listed the production of puparia reared from females from the 1st to 23rd day of oviposition in Generations 5 to 10. The age of the females may be calculated by adding three days to the day of laying in each case.

TABLE I

PRODUCTION OF PUPARIA FROM EGGS DEPOSITED BY FEMALES
THROUGHOUT THEIR EGG-LAYING PERIOD

Days of laying	Number of samples (jars)	Mean production of puparia per jar (gm.)
1	5	38.3
2	19	39.2
3	18	34.6
4	17	36.3
5	13	35.2
6	15	29.6
7	20	30.3
8	21	32.3
9	17	25.5
10	19	29.8
11	15	27.6
12	14	28.0
13	13	21.8
14	10	20.5
15	12	21.0
16	9	17.9
17	7	16.4
18	4	10.1
19	2	13.0
20	5	18.3
23	2	18.3

A similar drop in production with increased age of the females was also demonstrated in a strain referred to as 'wild', of which the original stock was secured from a barn near Belleville. Lower yields of puparia were due chiefly to decreased hatching of the eggs; as the females became older increased

proportions of the eggs failed to develop.   In Table II the mean proportion of hatched eggs calculated from 25 populations is shown for both strains. The drop in percentage of hatched eggs was greater in the Powell strain than in the 'wild' strain.

TABLE II

DIFFERENCES IN THE HATCHING OF EGGS AND THE AGE OF PARENTAL FEMALES

Day of laying	Percentage of eggs hatched		Day of laying	Percentage of eggs hatched	
	Powell strain	Wild strain		Powell strain	Wild strain
1	91.2	94.8	18	68.6	80.4
2	86.3	92.3	19	64 6	84.6
3	85.9	92.0	20	63.8	77.5
4	82.4	91.6	21	50.6	79.0
5	86.4	88.6	22	54.2	77.3
6	81 5	89.5	23	47.5	80.6
7	82.4	89.4	24	60.4	71.5
8	78.3	90.3	25	58.4	75.7
9	85.8	88.6	26	38.7	63.8
10	79.1	87.7	27	43.0	73.0
11	76.4	86.9	28	32.9	48.0
12	80 2	87.5	29	25.7	—
13	73.0	88 1	30	26.2	—
14	78.4	85.1	31	44.2	—
15	68.7	86.7	32	—	—
16	71.9	88.7	33	—	—
17	66.4	85.5	34	32.0	—

Since males have a shorter life than females, only a few were present in the cages on the 10th day of laying.   To determine whether the reduction in hatched eggs was due to the absence of males and a consequent deposition of sterile eggs, males were removed from several cages after mating had occurred once, while in other cages fresh males were introduced after 10 days of laying. The results showed that the decrease of percentage of hatch was not caused by the absence of male flies.   This conclusion was supported by observations made during egg sampling.   Unfertilized eggs could be recognized on inspection by their translucent appearance.   Such eggs were not more numerous towards the end of the oviposition period.   It seems probable that a change occurred in the nutrition of the eggs associated with increasing age of the females, although changes in the diet of females produced no significant differences in the rate of reduction of egg hatch with age of the parental females.

Lower yields of puparia were due also to a shortened life of some larvae as the age of the parental females increased.   The survival of larvae varied inversely with the days of laying.   In Table III a summary of production is given for two populations of adult breeding stock.   Eggs from these females were placed in rearing on each day of oviposition, at densities of 2500, 3000, and 3500 eggs per battery jar.   The jars were prepared in triplicate.

TABLE III

PRODUCTION RECORDS FOR THE COMPLETE OVIPOSITION PERIOD OF TWO ADULT
POPULATIONS OF POWELL FLIES

Period of egg deposition	Per-centage of eggs hatched	Production of puparia per jar			Survival of larvae, %		
		Eggs per jar					
		2500	3000	3500	2500	3000	3500
Generation P 16							
Mean 1 to 10 days	79.6	1670	1890	2100	83.6	79.1	75.1
Mean 11 to 20 days	72.4	1120	1310	1230	61.9	60.1	46.4
Mean 1 to 20 days	76.2	1430	1620	1720	73.8	70.2	62.6
Generation P 14							
Mean 1 to 10 days	85.5	1630	2070	2200	76.4	80.9	73.6
Mean 11 to 20 days	72.5	1040	1240	1420	57.5	57.5	56.7
Mean 1 to 20 days	79.0	1330	1660	1810	67.0	69.2	65.1

From these results it is concluded that variations in production of puparia between different populations of flies is often a result of differences in the age of the females but the rate of drop of production was not rapid until after the 10th day of oviposition. By this time between 60 and 70% of the total number of eggs had been laid. Thus by destroying the breeding stock after 10 days of egg laying variation in production was reduced but not completely eliminated.

Survival of Eggs

Samples of eggs to determine the percentage hatch were taken on all lots before rearing and thus provided a means of estimating expected production. Two or more lots of 100 eggs were counted and placed on damp, white blotting paper, soft paper towelling, or black photographic paper in a covered watch glass and incubated at 26.7° C. After 24 hr., the unhatched eggs were counted and subtracted from 100. Large series of tests showed that the standard deviation of individual samples was ± 4% and that there were no significant differences in hatch between the eggs taken for sampling and those hatching in the rearing medium. During the first 10 day period of egg-laying all viable eggs hatched within 24 hr. but after 10 days about 7% of the eggs did not hatch until after 24 hr. of incubation.

Within any one population of flies the decrease in hatching of the eggs with increased age of the females as shown in Table II was not regular from day to day. The curve is characterized by having fluctuations above and below the smoothed line, the magnitude of which increased with the age of the females. In the Powell strain the fluctuations occurred at daily intervals, a day of high egg hatch followed by one of low hatch. In the 'wild' strain the peaks appeared at approximately every three days. During the first 10 days of egg

laying the variations rarely exceeded 5%. Thus in the rearing program where a large number of jars were being prepared each day adjustments had to be made in order to provide for these cyclic variations in the hatching of the eggs.

Survival of Larvae

After the rearing medium has been 'seeded' with eggs it is not possible to follow closely the behavior of the larvae without disturbing the balance of conditions within the rearing jars. Estimates of larval survival were based, therefore, on a count of the puparia produced and the percentage of hatched eggs. No precise method of obtaining data upon the time of greatest larval mortality was devised, although under normal conditions, it appeared that most of the mortality occurred soon after the eggs had hatched, when the young larvae were becoming established.

Differences in survival of larvae occurred in much the same manner as in the case of the eggs, i.e. from one population to another and in daily cycles. The variations in survival of the larvae, however, were greater. It was also found that when the percentage of hatched eggs was unusually high the survival of the larvae was appreciably lower and vice versa. Thus, when the percentage hatch of the eggs was high some of the larvae were weakened and soon died and the proportion of less viable larvae decreased as the percentage of hatched eggs decreased.

The Rearing Medium

During incubation of the larvae active fermentation occurs in the rearing medium resulting in an almost continual change of chemical substances and the production of heat. Exact control of all the variables is almost impossible. In the present study it was found that the production of puparia was dependent to a large degree on both the quantity and quality of fermentation particularly during the early stages of larval development, and that unless some form of control was used the rearing was very unpredictable. On the other hand, apart from the necessity of using fresh yeast (can be held at 0° to 1° C. not longer than 10 days), varying the concentrations of the different ingredients composing the rearing medium had little effect on production of puparia.

The process of fermentation was influenced almost exclusively by temperature, aeration, and the water content of the rearing medium. Moisture was subject to more or less exact control by altering the amount of water used when preparing the medium and its effect on fermentation, therefore, was largely indirect. If the medium became too wet, aeration was poor and fermentation dropped, whereas if the medium became dry aeration was excessive and fermentation proceeded too rapidly.

Temperature and aeration, however, were not subject to such precise control, since they were both influenced by the amount of fermentation. The temperature of the rearing medium was the resultant of the heat of fermentation

and the loss of heat to the incubation chamber. Aeration was dependent on the wetness of the rearing medium, the amount of packing or settling of the medium in the jars, and the activity of the developing larvae in crawling through the medium.

A study was made of the temperature characteristics of the rearing medium in the jars as an index of fermentation. Recordings made from thermocouple points placed at different levels in the medium gave a general picture of the thermal changes in the medium from the first day of rearing until the puparia were formed. The results obtained from jars seeded with 2500 eggs and at a depth of 3 in. below the surface are shown in Table IV.

TABLE IV

TEMPERATURE CHANGES IN BATTERY JARS OF
REARING MEDIUM INCUBATED AT 26.7° C.

Days of rearing	Temperature in ° C.
One, just after seeding eggs	32
One, six hours later	35
Two	42
Three	45
Four	44
Five	43
Six	36
Seven	32
Eight	30
Nine	29

Although both vertical and horizontal temperature gradients were constantly present, except for those at the immediate surface and circumferential layers, they rarely exceeded 3° C. As can be seen in the table, the temperature of the medium increased until the third or fourth day of rearing after which it began to decrease. The maximum temperature in these lots was 45° C. This temperature appeared to be somewhat critical since above this range serious crowding of the larvae ensued. Developing larvae had a definite temperature preference of 40° to 42° C. and would, therefore, migrate to the lower temperatures at the peripheral and surface layers where crowding would result in excessive mortality and the formation of abnormal puparia.

When fermentation was poor and the temperature of the medium did not exceed 36° C. development was prolonged by two to three days and survival of the larvae and production of puparia was low. The medium also remained

wet and tended to pack into sticky masses making removal and separation of the puparia very difficult.   The effects of various temperatures of the medium on puparial production are shown in Table V.

TABLE V

DIFFERENCES IN PUPARIAL PRODUCTION AT DIFFERENT
TEMPERATURES OF THE REARING MEDIUM

Experimental number	Normal temperatures	Abnormal temperatures
Generation 18-3		
Highest recorded temperature	41° C.	47° C.
Number of puparia per jar	1650	1290
Survival of larvae, %	76.7	59.9
Generation 32-2		
Highest recorded temperature	41° C.	46° C.
Number of puparia per jar	1540	1510
Survival of larvae, %	78.6	77.0
Generation 25-3		
Highest recorded temperature	42° C.	36° C.
Number of puparia per jar	1750	1080
Survival of larvae, %	87.5	54.0

Various attempts were made to control the rate of fermentation in the battery jars and thus decrease the possibility of variation in production. Aeration of the rearing medium was regulated to some extent by avoiding packing when filling the jars and by seeding with a constant number of eggs. Variations in aeration were chiefly due therefore, to the differences in larval activity.   During the first day or two of rearing, young larvae were evenly distributed throughout the medium.   As the temperature of the center of the medium rose to 42° C. and above, the larvae migrated to the surface layer where their feeding and movements made the medium loose and finely divided, allowing access to the air.   This normally occurred on the third day.   As incubation continued, the larvae fed at greater depths bringing air to the mass of medium lower in the jar.   Aeration could only be controlled, therefore, by preventing the formation of a crust of medium in the upper layer before the larvae had an opportunity to feed at the surface.   This was accomplished by covering the jars with damp towels for at least the first three days of incubation.   In battery jars, under normal conditions of rearing, control of the temperature was almost impossible and could only be attempted by regulating the room temperature within rather narrow limits of from 24° to 28° C.

To provide a more precise means of maintaining temperature control and thus decrease variations in puparial production, wooden boxes were used very effectively for rearing.   These were fitted with copper tubing through which water was circulated at a constant temperature throughout the medium. Using these rearing units surprisingly uniform production was obtained.   In an experiment designed to compare the production of puparia in medium at

constant temperatures 5° C. higher and 4° C. lower than the normal range of rearing as shown in Table IV, no significant differences in the number of puparia were observed, although at the higher temperatures a greater number of smaller puparia were formed. This is shown in Table VI.

TABLE VI

PUPARIAL PRODUCTION IN BOXES FITTED WITH TEMPERATURE-CONTROL COILS

	Puparia from medium equivalent to one battery jar	Survival of larvae, %	Weight of puparia (per 1000)
Mean at normal temperature	1650	84.7	19.258
Mean at 5° C. above	1518	78.3	17.561
Difference	132	6.4	1.696
Number of tests	5	5	5
P values	0.1	0.1	0.05
Mean at normal temperature	1554	85.3	17.930
Mean at 4° C. below	1568	86.2	18.122
Difference	−14	−0.9	−0.192
Number of tests	5	5	5
P values	>0.5	>0.5	>0 5

Density of Larval Population

The optimum density of larvae in the rearing jars was obtained by seeding them with 2500 eggs. At this density and under standard rearing conditions from 1600 to 1800 puparia were recovered. At higher densities the survival of larvae was much lower, particularly, as in many instances, where the temperature became abnormally high. Although it was found possible to rear flies at densities up to 7500 eggs per jar, survival of the larvae was very low and the production of puparia much reduced.

Using wooden boxes in which temperature of the medium was under constant control, a series of 10 tests was made to compare production of puparia at densities of 2500 and 5000 eggs per quantity of medium equal to one battery jar. In each test six boxes were seeded with 2500 eggs per jar of medium and in six others twice the number of eggs were used. The results are shown in Table VII.

TABLE VII

PRODUCTION OF FLY PUPARIA AT DIFFERENT DENSITIES OF EGGS

	Number of puparia per jar of medium	Survival of larvae, %	Weight of puparia per 1000
2500 eggs per jar of medium	1795	88.4	18.395
5000 eggs per jar of medium	3165	78.2	14.139
Mean difference	−1370	10.2	4.256
Number of observations	10	10	10
P value	<0.01	<0.01	<0.01

From the statistics shown in the table it is clear that crowding of the developing larvae has marked effects on the production of puparia. This is expressed in a reduction of the number of puparia produced from each egg (from 72 to 63%) and/or as an increase in the mortality of the larvae and a 25% decrease in the size (weight) of the puparia.

Differences Between Lines of Breeding Stock

Differences in the production of puparia were apparent not only between different populations but between different lines of breeding stock. The constantly high production of certain selected lines was of considerable value in the production of a uniform and predictable quantity of puparia.

Early in the studies a series of selection experiments was carried out beginning with the 18th generation of laboratory reared flies. A number of pure bred lines were established, in which selection was maintained on the basis of puparial production per battery jar of medium. The number of eggs used was the same throughout. Selection was continued until the 30th generation. At that time no further improvement was evident and the most productive line was used to replace the general breeding stock. A summary of puparial production for five of the selected lines and the general laboratory stock is given in Table VIII. The statistics shown represent the mean values calculated from at least 10 daily lots from each of the generations indicated under the different lines.

TABLE VIII

PUPARIAL PRODUCTION FROM DIFFERENT LINES OF BREEDING STOCK

	Puparia per jar		Survival of larvae, %		Weight of puparia (per 1000)		Number of genera- tions
	Mean	σ	Mean	σ	Mean	σ	
General laboratory stock	1600	300	82 8	12 5	19 32	1 50	47
Line A	1750	255	87 2	10 3	18 58	1 50	13
Line B	1430	237	76 9	11 9	20 00	0 87	7
Line C	1440	254	78 6	13 5	20 14	0 92	11
Line D	1820	154	93 1	6 0	19 51	1 14	7
Line E	1130	332	62 7	13 3	18 76	0 76	2

From a comparison of the lines, both *A* and *D* gave significantly greater production than any of the others and Line *D* was the most productive of all with respect to the number of puparia and the survival of the larvae. It is also noticeable that the variance in the *D* line was much lower than in any of the others. The differences in size of puparia were not significant.

Using only flies from the *D* line as breeding stock large scale production of puparia was carried out for two years in a very uniform and predictable

manner. From the production records through 88 generations and on occasions at the rate of almost one million puparia per day the following constants were obtained.

Mean number of puparia per jar of medium	1770	± 240
Mean survival of eggs placed in jars, %	70.9 ±	9.3
Mean percentage of eggs that hatched	80.1 ±	5.1
Mean survival of larvae, %	87.5 ±	8.6

In obtaining these constants, calculations were based on propagation records from only the first 10 days of egg deposition and under rearing conditions to be described later in which 2500 eggs were used per battery jar of rearing medium.

Differences in Laboratory Populations of Flies

Populations of flies when used as breeding stock or as test animals in experimental studies showed considerable variation with respect particularly to the percentage of adult emergence from the puparia, the sex ratio, length of adult life, fecundity, and the periods required for adult emergence and egg deposition. The variations in length of life and fecundity were greatest, although the influence of all had to be considered in the routine rearing and testing of the flies.

Adult Emergence

When puparia were incubated immediately following separation from the rearing medium at 26.7° C. and 50% relative humidity little variation in adult emergence occurred. Under these conditions the first adults appeared in 3 ± 0.5 days and nearly all the puparia produced normal flies. The mean adult emergence from puparia reared through 40 generations was 92.6 ± 2.4%. If, however, the period between the formation of the puparia and emergence of the adults was prolonged particularly by lower temperatures both the number and rate of adults emerging was considerably altered and became very variable. A detailed account of experiments with puparia at low temperatures is given in a later paper of this series.

Sex Ratio

Although the sex ratio of the emergents varied somewhat from one group of flies to another in most samples containing over 10,000 it was remarkably constant. The mean sex ratio (percentage of females) of flies reared in 40 generations, representing over one million individuals, was 50.0 ± 5.0. In small samples the sex ratio varied from a minimum of 40 to a maximum of 60% females depending on when the sample was taken. Since males normally emerge before the females, samples taken early in adult life will contain a preponderance of males, while if the sample is taken after three days of adult life there will be a greater proportion of females, since males have a much shorter life than females.

Length of Adult Life

Considerable variation in length of adult life was noticed between different populations of flies. Individual differences within a population were even more striking. Males began to die three to four days after emergence. In breeding cages a few males were dead on the first day of oviposition and most of the males had died by the 10th day of laying, although some lived for as long as 20 days. Death of the females began on about the third day of egg laying (six to seven days old), about one-third being dead after 10 days of oviposition. A very small proportion of the females lived for a maximum of 40 days. The mean length of life of flies taken from generations 20 to 60 was, for males 12 $\pm$ 2 days and for females 20 $\pm$ 2 days. The percentage dead in the breeding cages after 10 days of oviposition was, for males, 71.1 $\pm$ 19.3 and for females, 31.6 $\pm$ 13.0.

In order to obtain a constant and predictable supply of eggs, care had to be exercised in guarding against excessive female mortality. This was done by keeping the breeding cages liberally supplied with dishes of skimmed milk and water. Attempts were made to prolong the life of flies by feeding them whole milk at various dilutions as well as milk with malt and yeast, or both, but there were no significant differences either in mortality or fecundity.

Fecundity

The greatest variable encountered in rearing the flies was in the number of eggs deposited by the females. In determining the number of flies required for the propagation of a predictable number of puparia certain empirical corrections always had to be made. Experiments in which individual flies were used showed that many of them laid no eggs. It was found, however, that isolated flies rarely produced a normal number of eggs and few laid more than 200. In larger groups of adults, egg production for cages of 500 flies (the average for 40 generations) was 500 $\pm$ 100 per female, 65% of which were laid in the first 10 days of oviposition. The rate of oviposition was 30 $\pm$ 10 per female per day for the first 10 days. After that time egg deposition decreased rapidly and at an irregular rate.

In all populations of flies the greatest production of eggs occurred on the second or third day of oviposition, and decreased as the flies aged. Even before the 10th day oviposition was characterized by having daily fluctuations, a day of high egg deposition being followed by one of low deposition and vice versa. Some of these variations were caused by differences in the mortality of females and by the escape of females from the cages when food dishes were changed. Even under carefully controlled experimental conditions appreciable variation occurred. A number of possible causes were investigated, including size of the adult, type of food, the temperature and population density at which the flies were reared, the days on which eggs were laid, and selection of breeding lines. No significant correlations were obtained from the data secured in 53 replicate samples through 13 complete generations.

Time of Emergence and Oviposition

Differences in the time between removal of the puparia from the rearing jars, adult emergence, and subsequent egg deposition depended almost entirely on the stage of puparial development at the time the puparia were separated from the medium. When rearing was done in battery jars the puparia were removed on the morning of the ninth day, i.e. after eight complete days of incubation. At 26.7° C. 140 hr. were required between the formation of puparia and adult emergence. Females required approximately three hours longer than males. Puparia, when removed from the rearing jars had usually completed an average of about 70 hr. of development and approximately half the puparia had undergone 65 to 95 hr. of development. Thus the amount of development, being a function of the temperature of the rearing medium, had a marked influence on the time of emergence and oviposition.

In wooden boxes where the temperature of the medium could be accurately controlled, differences in the age of the adult stock within comparable populations were very much reduced. Under these conditions puparia were removed after seven complete days of rearing. At that time the mean age of the puparia was 70 hr., but over 80% were within the age class of 65 to 95 hr. of development and over 80% of the adults would emerge within a 24 hr. period.

Differences Between Wild and Powell Strains

For the most part, in the experiments carried out during the present study the flies were propagated from a selectively-reared 'Powell' strain used almost exclusively in Peet-Grady tests. In order to compare the results with those from wild populations 40 generations of flies reared from a stock of adults captured near Belleville were propagated in a similar manner and under the same incubation conditions.

From the records of production and adult behavior it was found that the wild strain had variations similar in almost every respect to the Powell strain and that the differences between the two strains were very slight. No significant differences were found in the percentage of adult emergence, sex ratio, rate of development and oviposition, or in fecundity. Although it was noticeably more difficult to induce the wild females to lay their eggs and in most cases it was necessary to mix a little of the rearing medium with the milk in the oviposition dishes, the total number of eggs deposited was essentially the same. The wild flies lived a little longer than the Powell flies, the mean length of life of the females being four days longer; the life of the males was of similar duration. The greater mean duration of life of the wild flies was due largely to the presence of a greater proportion of individuals that lived far past the normal life span. The maximum longevity of wild flies in cages was 50 days.

Under favorable conditions of rearing, particularly with respect to fermentation, greater larval mortality occurred in the wild strain. It appeared that the adaptability of larvae from the wild strain was less than larvae from the

Powell strain and that under unsuitable conditions the Powell stock was more productive of puparia.   A greater number (about 3%) of the eggs deposited by the wild strain produced larvae but fewer of the Powell larvae died during rearing so the production of puparia was the same.

Methods Adopted for Large Scale Production

For the handling and propagation of the large quantities of flies required, the technique of rearing outlined under general methods of rearing (p. 9) was completely inadequate.   By following the usual technique the space and time required for rearing were far beyond the scope of available facilities and, in addition, were a source of considerable variation with respect to both production and the behavior of the flies during tests.   Studies were, therefore, undertaken with a view to devising more suitable methods of propagation. After considerable experimentation a procedure was finally adopted that eliminated most of the undesirable features of the previous method and yet made it possible to rear on a predictable and continuous basis millions of flies per day.   An outline of the methods used is given under the headings listed below.

Handling Adult Flies During Mating and Egg Deposition

The cages used to hold the flies for mating and oviposition were made up as required from demountable wooden units fastened together to form a rect-angular structure 3 × 3 × 3 ft.   Each of the four sides as well as the top and bottom consisted of a screen covered section that, when assembled, could be fastened to its neighbors by carriage bolts and wing nuts.   Thus the cages could be readily dismantled for cleaning.   Across one side were three rows of paired wooden doors, permitting access to the cage.   The edges of the doors were lined with weatherstripping to prevent escape of the flies.   From the bottom of each door to the opposite side of the cage were placed two movable wooden strips separated by a distance equal to the width of a cafeteria tray. The trays, of enamelled metal 17 × 12 × $\frac{3}{4}$ in. had an overhanging edge by which they were held in place on the wooden strips.   Six trays were used in each cage.

Before being placed into the cages the trays were filled with a milk solution consisting of equal parts of skimmed milk and water.   Each tray held about one pint of milk solution.   The surface of the fluid was covered with paper towelling supported on small blocks of cork and a small crumpled piece of towelling laid on top.   Most of the eggs were deposited upon the relatively drier surface of the upper piece of towel.   Cages set up in this manner provided approximately 70 sq. ft. of roosting surface and would satisfactorily accommodate from 10,000 to 20,000 flies.

Each morning the trays of milk were removed and replaced by fresh trays. The eggs were washed from the towelling into a jar of water, using a fine spray nozzle.   The egg suspension was passed through a 14 mesh screen to remove dead adults and large milk clots and then into an 80 mesh screen container

where the eggs were washed free of milk particles with a fine spray. The eggs were later resuspended in water and poured into a glass tube $\frac{1}{2}$ in. in diameter, capped on the bottom with a removable piece of 80 mesh screening. The number of eggs could be estimated within a maximum error of $\pm 4\%$ by a calibration of the tube. One inch of the tube held 16,000 eggs, provided at least a 2 in. head of water was maintained in the tube above the eggs.

From each of 15 populations of 10,000 flies handled in the above manner, two samples of 500 flies were withdrawn and treated according to the usual Peet-Grady methods. Counts of egg deposition and hatch were made on this series of paired populations and the results analyzed by the 't' test for paired variates. A small but significant difference was found in the hatching of the eggs; 84.5% of the eggs from the small cages produced living larvae, while only 80.4% of the eggs hatched from the large cages. Apparently a greater proportion (4%) of eggs was injured during cleaning and measuring from the larger cages. Differences in fecundity were very small and in no cases were they statistically significant.

Larval Rearing Containers

A number of different types of rearing containers were constructed and tested in large scale puparial production. For moderately large propagation (from 100,000 to 300,000 puparia per day) the best larval rearing container was a wooden box of the dimensions $16 \times 11\frac{5}{8} \times 6\frac{1}{2}$ in., constructed of $\frac{7}{8}$ in. cypress or cedar in which could be placed the equivalent to four battery jars of rearing medium. The box was sealed on the inside with a coating of hard paraffin and beeswax. Each box was fitted with a set of copper coils by which the temperature of the medium was constantly controlled. The coils were made up from $\frac{5}{8}$ in. copper tubing 5 ft. long, bent into a circular pattern and placed on small blocks one inch from the bottom of the boxes. The coils were joined to a water conduit system equipped with hand operated valves, a centrifugal pump, and a reservoir in which the water was maintained at a constant temperature of 36° C.

The use of the water circulating system provided an excellent means of controlling fermentation and it was thus possible to greatly reduce variations between different units or lots. This was of particular value in reducing the spread in ages of the puparia and in eliminating excessive larval mortality associated with abnormally high temperatures. Production of puparia was as high as in the battery jars. The mean number of puparia produced per box seeded with 10,000 eggs during 40 generations of continuous propagation was 7080.

A number of other types of rearing containers were tried with varying degrees of success. Of the different types used, biscuit tins holding four battery jars of medium, galvanized iron trays holding 60 jars of medium, and wooden trays holding 144 jars were the most satisfactory. In general, as the

size of the rearing container was increased, the mortality of the larvae increased and the puparia produced were smaller, owing to the development of high temperatures in the larger masses of medium. In metal containers, where the heat accompanying fermentation was dissipated rapidly, puparial production was equivalent to that in battery jars but the metal corroded very rapidly and soon had to be replaced. In the large wooden trays temperatures rose as high as 60° C. and it was, therefore, essential to equip them with a cooling device made up from copper pipe operating through a pump and water conduit system. By regulating the temperature and flow of the water and by limiting the depth of the medium to 6 in., conditions within the medium could be controlled sufficiently to produce puparia comparable in number and size to those from battery jars and the small wooden trays. The average production of puparia per large wooden tray was 250,000.

Rearing Medium

In order to avoid the laborious task of mixing by hand a machine was constructed to handle the greater quantities of medium required in large scale rearing. The mixer was made up by mounting a 50 gal. oak barrel on a horizontal axle and was revolved by means of a ¼ h.p. electric motor and a series of differential pulleys. A door 9.7 in. wide made by removing several staves along one side was held in place by heavy spring clamps and fitted tightly against a rubber gasket. Up to 48 jars of medium could be mixed at one time.

For the most part, the medium used for rearing larvae was mixed 24 hr. before being seeded with eggs. Medium stored at room temperature for two days in battery jars was satisfactory for rearing. When the large temperature-controlled boxes were used, freshly prepared medium seemed to give best results.

During most of the rearing the modified Peet-Grady formula outlined on p. 9 was used in making up the medium. At times during the course of the work, alfalfa meal could not be found in sufficient quantity and it was necessary to try substitutes. After experimenting with a number of substances it was found that the larvae could be reared in a medium in which all or part of the alfalfa meal was replaced by sawdust. Medium sized sawdust gave best results. Sawdust could not be substituted for any part of the bran.

The use of sawdust as a substitute for alfalfa in the rearing medium had a marked effect on the production of flies. The yield of puparia was considerably lower and there was a noticeable reduction in the emergence of adults from the puparia. The adults that did emerge, however, although somewhat smaller in size, reacted the same in experimental tests as individuals reared on normal medium. In Table IX a comparison is made between paired populations of flies reared on standard sawdust media. The differences are statistically significant in the first five columns and not significant in the last two.

TABLE IX

DIFFERENCES BETWEEN FLIES REARED ON STANDARD MEDIUM AND MEDIUM IN
WHICH SAWDUST WAS SUBSTITUTED FOR ALFALFA MEAL

	Puparia per jar of medium	Survival of larvae, %	Survival of eggs, %	Weight of puparia (1000)	Emergence of adults from puparia	Eggs per female per day	Adult mortality in first 10 days, % of total
Standard medium	1592	80.8	63.6	19.41	93.0	23.0	54.3
Sawdust medium	1402	70.3	56.1	14.35	81.6	22.9	59 1
Mean differences	190	10.5	7.5	5.06	11.4	0.1	−4 8
Number of observations	5	5	5	5	10	5	5
P value	0.03	0.04	0.03	0.02	<0.01	>0.5	0.2

Separation and Treatment of Puparia

To dry and separate the large number of puparia from the medium a wooden-framed, masonite tower 2 X 2 X 6 ft. was found to be very useful. Twelve wooden-framed, screen bottom trays, 3 in. high, were placed in the tower and the air drawn through them by means of a high speed exhaust fan mounted on the top. Each screen tray held the puparia and upper layer of four jars of medium. The contents of 12 trays (100,000 puparia) was dry and ready for separation at the end of an hour.

Separation of the puparia from the dried medium was accomplished in a very short time by passing the mixture through an electrically-driven grain seed or bean cleaner. Using this winnowing machine 500,000 puparia could be thoroughly cleaned and separated from all waste in one hour.

Separation of the puparia is also possible by repeated washing in water. The puparia float on the surface of the water and can be readily skimmed off the top. These puparia are particularly free from adhering waste particles and require no previous drying.

References

1. MacNair-Dorland Company. Blue book. *Issued by the publishers of* Soap and Sanitary Chemicals, N.Y. 1943.
2. Richardson, H. H. An efficient medium for rearing houseflies throughout the year. Science, 76 : 350-351. 1932.

STUDIES ON THE HOUSEFLY (*MUSCA DOMESTICA* L.)

II. THE EFFECTS OF LOW TEMPERATURES ON LABORATORY-REARED PUPARIA[1]

By G. E. Bucher,[2] J. W. MacB. Cameron,[3] and A. Wilkes[4]

Abstract

Investigations are reported on factors influencing the survival of housefly (*Musca domestica* L.) puparia exposed to different intensities of low temperature storage above 1° C. Survival was decreased by lowering the temperature of storage and by increasing the duration of the storage period, or both. Mortality of adults following puparial storage was influenced by the type of rearing container used, crowding, age of puparia, humidity, concentration of gases in the pupal rearing chambers, and to some extent by changes in the food of the immature larvae but not by the age or sex ratio of the parental stock, the size of puparia, selective breeding of resistant individuals or different strains of stock. Death did not occur in cold storage but during subsequent incubation at normal temperatures and at a definite stage in development near adult emergence. Temperatures below the threshold of development caused physiological disturbances that affected the longevity, oviposition, and hatchability of eggs of the adults that survived. A proposed explanation is given of the lethal effects of low temperatures based on the interrelationships of disturbances between the relative rates of development and differentiation of various ontogenetic systems.

Introduction

The importance of temperature as a factor governing the lives of insects and other poikilothermic animals is demonstrated by the vast literature, surveys of which have been made by Payne (12), Uvarov (21), and Bélehrádek (3). Entomologists have been concerned chiefly with the influence of temperature upon the rate of metabolism or the speed of development and with considerations of the factors by which insects are able to survive periods of extreme cold.

Although a number of authors observed that the time of exposure might affect the survival of cold-hardy insects in freezing temperatures, little attention has been paid to a general study of the "quantity factor" referred to by Payne (13). Studies of the effect of prolonged exposure to temperatures at which protoplasm does not freeze have been confined to the insects of stored grain (*vide* Back and Cotton (1); Larson and Simmons (10); Carter (6), Robinson (17)). There is consequently little information available on the effects of prolonged exposure to moderately low temperatures and there is no universal agreement as to the cause of death in insects that are not resistant to this treatment.

The present investigation upon the resistance of housefly puparia to cold and the physical and biological factors that influence survival was undertaken to determine the possibility of building up large populations by continuous propagation and low temperature storage. Although the experimental evidence demonstrated that this was not practical and cast doubt on the ability of houseflies to overwinter as puparia, the data obtained are of value in providing further information on the reactions of common insects to the so-called quantity factor. The experimental evidence presented in the present study supports the theory of development suggested by Powsner (16), as an explanation of the lethal action of temperatures above the freezing point of protoplasm.

This paper was taken from a dissertation presented by the senior author (Bucher) in partial fulfillment of the degree of Doctor of Philosophy at the Ohio State University. For tables of original data and a more detailed statistical analysis, reference may be made to the dissertation deposited in the library of the Ohio State University.

Methods of Experimentation and Analysis of Results

The fly puparia used in the experiments were reared by a modification of the Peet-Grady method, an outline of which is given in Part I of this series.* Samples of 500 puparia were withdrawn at random from well-mixed populations, placed in storage containers and exposed to various periods of low temperature. At the conclusion of the storage treatment, the puparia were incubated at 27.6° C. until emergence of the adults had ceased. The adults that emerged were counted, checked for errors by a count of unemerged puparia, and the number expressed as percentage emergence or percentage survival.

The resistance of a puparium to cold storage was, therefore, measured by an all-or-none criterion, the emergence or nonemergence of an adult fly after return to the incubation temperature. Individual variations in resistance occurred between puparia of a single population, reared and stored in an identical manner, so that, after any given treatment, part of the population succumbed while the remainder survived and produced adults. By the use of a sample of puparia and the calculation of percentage survival, a measure of the resistance of puparia to storage treatment was obtained. If samples were withdrawn from a population of puparia and subjected to different intensities of storage, or if samples were withdrawn from different populations and subjected to the same intensity of storage, differences in survival were obtained. It was therefore necessary to determine what proportion of these differences in survival was due to experimental treatment or to

* *Wilkes, A., Bucher, G. E., Cameron, J. W. MacB., and West, A. S.   Can. J. Research, D, 26: 8-25.   1947.*

innate differences between populations, and what proportion was due to chance, in order that the significance of variations in survival of samples could be assessed.

It was found by experiment that, if a series of samples of 500 puparia were withdrawn from a well-mixed population and subjected to identical storage conditions, the percentage survival of any one sample rarely varied from the mean of the series by more than 2.5, while the difference between the highest and lowest samples rarely exceeded 5.0. This result conformed closely to that predicted by the law of random variation of percentages in which $s = \sqrt{\frac{p.q}{n}}$, where p is the population parameter (i.e. in this case the true survival of the population), q is 100-p, n is the number of individuals in the sample, and s is the standard deviation of the statistic (in this case the expected deviation in percentage survival between samples caused by chance alone). If p equals 50%, for a sample of 500, s becomes 2.2%. That is, within a series of samples treated alike, about two-thirds of the samples would show survivals within 2.2% of the mean and be within a range of 4.4%.

In the experiments two samples of puparia were used for each treatment and the percentage survival calculated for each separately. When the survivals varied from each other by 5% or less, the mean was accepted as representing the best available measure of the population parameter. If the survivals varied by more than 5% (a rare occurrence), the records were discarded as not reliable. The mean survival figures for a given treatment and population are considered as accurate to within 2.5%. Theoretically, as the population parameter approaches 100% or 0%, the standard deviation decreases and smaller differences may be regarded as significant. In these studies, however, a difference of less than 2.5% was regarded as not significant at any value of the parameter.

The validity of the sampling technique and the use of the equation $s = \sqrt{\frac{p.q}{n}}$ to estimate the standard deviation due to chance was based upon the hypothesis that the fly population was normally distributed and that no large components of the population were inherently more resistant than others and thus predisposed to survival. In order to test this hypothesis and to determine whether resistance to cold was inherited, a series of selection experiments was carried out.

A population of 70,000 puparia was stored at 6.0° C. for 15 days. The surviving adults, numbering about 1%, were bred and their progeny reared, the puparia being stored at 6.0° C. for 10 days. Surviving adults were again bred and the puparia from the filial generation stored at 6.0° C., for 10 days. This procedure was repeated for seven generations.

Survival of puparia did not increase as the selection was continued. After seven generations survival of the selected population was compared with a population of equal age from the normal breeding stock (Table I). There was no significant difference in survival.

TABLE I

SURVIVAL OF PUPARIA FROM SELECTED AND NORMAL
POPULATIONS AT 9.4° C.

Days of storage	Percentage survival of puparia	
	Selected strain (mean age 68 hr.)	Normal strain (mean age 70 hr.)
0	93.2	94.2
3	93.6	92.8
5	87.2	90.8
10	70.0	69.8

Selective breeding of the portion of a population surviving puparial storage over seven generations did not increase the resistance of puparia to storage. Thus it is concluded that selection did not produce a change in cold resistance.

Puparia from some populations displayed considerably more resistance to cold than those from other populations. The most important of the factors causing these differences in resistance were the temperature of rearing and the age of the puparia when stored. In order to obtain reliable data from which to determine the amount of resistance of puparia subjected to various storage treatments, it was necessary to reduce variations between populations caused by differences in age, temperature of rearing, and other factors, and devise a rearing technique whereby puparia of maximum and uniform resistance could be produced in numbers. Puparia reared in this manner were designated as 'normal'. Normal puparia in this case were those reared at a density of 2500 eggs per jar unit of standard rearing medium in temperature-controlled boxes or in battery jars in which the temperature cycle followed a normal pattern and the maximum temperature did not exceed 42° to 44° C.

At the commencement of storage treatment using normal puparia about 80% were within the most resistant age group, while nearly all were within the range of 40 to 110 hr. The mean age of any experimental population was estimated by dissection or calculated from time-emergence records of untreated samples.

A small number of newly-formed puparia or puparia close to emergence was present occasionally even in a normal population. When experimental samples were withdrawn these were recognizable by color and rejected, as well as puparia with injuries or other abnormalties. The number of rejected puparia never exceeded 1%. With these exceptions, samples were withdrawn at random from well-mixed populations.

Temperatures of 1.0°, 6.0°, and 26.7° C. were secured in temperature-controlled chambers, having an accuracy of ± 0.5° C. Other temperatures were obtained in a multiple constant-temperature cabinet that operated at an accuracy of ± 0.1° C.

The flies used for the most part in these studies were reared from the Powell stock. A wild strain was propagated from flies collected at Belleville. Parallel

tests, conducted on both strains, indicated that there was no significant difference in their resistance to low temperatures. Therefore, while the data here reported are based upon a strain of flies of considerable genetic homogeneity, the results are equally applicable to wild flies.

The Influence of Age of Puparia on Survival

It has long been recognized that age has an important influence upon the resistance of both plants and animals to chilling. In insects, some developmental stages are more resistant than others (Bodine (5); Ludwig (11)). Within any given developmental stage, age may also have an effect on resistance as shown for ant pupae by Pictet (15). In the housefly, where the pupal stage is relatively short and apparently includes no diapause, an enormous amount of metabolic activity must occur. It was likely, therefore, that at certain periods in metabolism, the organism would be more susceptible to unfavorable temperatures than at others. To test this hypothesis, puparia of known age were subjected to a period of five days' storage at 6.0° C.

The results obtained from the series of experiments are shown in Table II. Age was measured in hours at 26.7° C.

TABLE II

VARIATION IN RESISTANCE TO COLD STORAGE WITH AGE OF PUPARIA (FIVE DAYS AT 6° C.)

Limits of age of puparia when stored	Mean age of puparia	Sample size	Emergence (survival), %
0- 1 hr.	0 hr.	397	12 6
1- 2	1	136	1.5
2- 3	2	123	14.6
3- 4	3	299	23 1
4- 5	4	293	31 1
5- 7	6	182	13 2
7- 9	8	268	11 6
9- 11	10	282	10.3
11- 13	12	247	9.3
13- 15	14	178	8 4
15- 17	16	219	0.9
17- 19	18	144	0 0
19- 21	20	207	5.3
21- 23	22	733	16 6
23- 25	24	768	34.8
25- 35	30	2098	64 2
35- 45	40	1851	66.4
45- 55	50	1657	73.9
55- 65	60	1299	77.1
65- 75	70	1140	82.1
75- 85	80	885	79.6
85- 95	90	1293	83.3
95-115	105	825	74.7
115-135	125	833	73.5
135-140	Emergence begins		
Control	No storage treatment	762	85.4
Mean emergence 65-95 hr.			81.7

Puparia less than 30 hr. of age at 26.7° C. were very susceptible to killing in cold storage.   Resistance increased slowly with age above 30 hr., reached a maximum at 90 hr., and thereafter decreased slowly.   At 26.7° C., between 135 to 140 hr. of development were necessary for pupation, females usually requiring one to two hours longer than males.   The curve of resistance as influenced by puparial age is shown in Fig. 1.

AGE   HOURS AT 26.7°C.
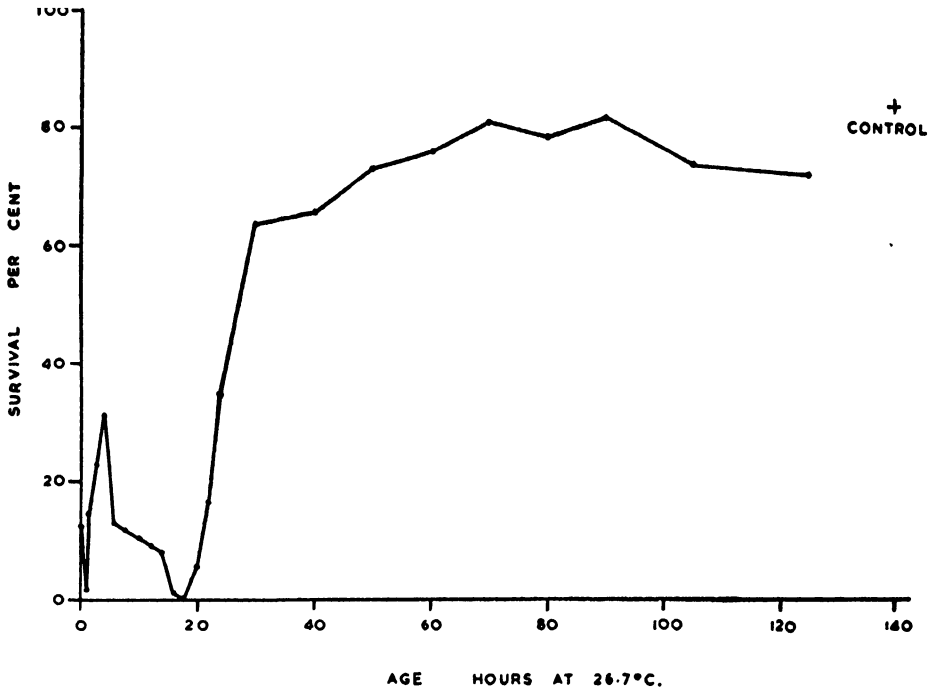
Fig. 1.   *The survival of puparia of different ages exposed to 6° C. for five days.*

Since puparia were most resistant to cold temperatures when stored after 65 to 95 hr. of development at 26.7° C., attempts were made to use, for further experiment, populations in which the majority of the puparia were within this age group.   In order that the age of the population could be estimated before experimenting, a brief study of the most evident morphological changes during pupal development was undertaken.

Between 0 and 12 hr. of development at 26.7° C. puparia change from cream to buff to red, and thus age can be recognized by inspection.   The interior is wet and superficially disorganized.   The head region is larval in appearance, is fastened to the puparial wall, and will pull off with the first segment. The mouth hooks are intact and there are tracheae attached to the anterior spiracles.

Between 12 and 24 hr. the head becomes more clearly defined, the antennal areas being plainly visible.

After 48 hr. the body begins to assume an adult shape and is largely free from the puparial wall.   The mouth hooks are free of the head and can be

found in the anterior portion of the puparial case. Tracheal connections to the anterior spiracles of the puparium have disappeared. The antennal areas become buff colored. Leg and wing buds can be seen.

At 60 to 70 hr. body formation is more like the adult. The antennal areas become brownish in color.

Between 70 and 95 hr., the head assumes a definite adult shape. Compound eyes are distinct. They are white in color but gradually turn to amber at the close of the period. The ocellar spots begin to darken.

Between 95 and 120 hr. the compound eyes become rusty brown. The body begins to harden and darken. The appendages are well formed. The legs become fuscous.

Between 120 and 135 hr. the puparia turn black and the compound eyes red. The insect has the form and coloration of an adult.

By use of these rough morphological characters, the mean age or stage of development could be determined by dissection of a sample of the population. The most resistant stage occurred when the puparia had brownish antennal areas and distinct compound eyes, either white or amber in color.

Survival of Puparia at Different Intensities of Cold Storage

Variation in the intensity of low temperature treatment may be secured by varying either the storage temperature or the duration of the storage treatment. During the course of this study experiments were carried out to determine survival under different intensities of storage.

After the storage treatment, the samples of puparia were placed in open Petri dishes within screened cages at 26.7° C. until emergence had been completed, at which time survival was assessed.

When survival was plotted against the period in storage, a series of curves was obtained, each curve representing a different temperature of storage. It can be seen from the slope of these curves (dotted lines in Fig. 2) that about 10% of the puparia are considerably more resistant to storage than the remainder. Since the mean emergence, calculated from a large series of observations, was $92.6 \pm 2.4\%$ for unstored puparia it also can be seen that approximately another 10% of a normal population of puparia are extremely sensitive. Between these two extremes they exhibit varying degrees of resistance to cold storage. The relationship between percentage survival and period of storage appears to be parabolic in form, when the very resistant and very susceptible components of the population are disregarded.

In order to demonstrate the relationship between survival and the period of storage and to make an estimate of survival for any particular period of storage, equations were fitted to the data by the method of multiple regression described by Snedecor (20).
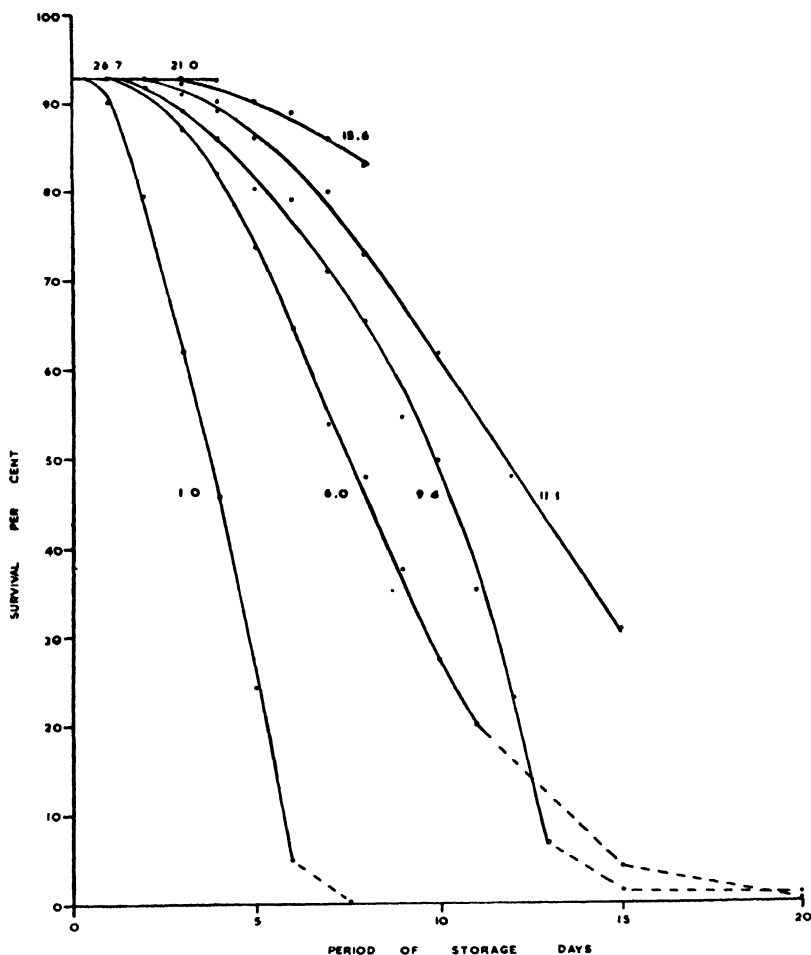
FIG. 2. *Survival of puparia following various lengths of exposure to low temperatures.*

Good fits were secured by the use of the polynomials of the form $Y = a + bX + cX^2 + dX^3$, where Y is the percentage survival, X the period of storage in days, and a, b, c, and d are constants specific for the temperature of storage. The equations are listed below.

Temperature	Equation	R
1.0° C.	$Y = 102.16 - 9.6697X - 1.465X^2 + 0.00335X^3$	0.99885
6.0° C.	$Y = 90.90 + 3.4660X - 1.7510X^2 + 0.07734X^3$	0.99745
9.4° C.	$Y = 98.09 - 4.3722X + 0.42165X^2 - 0.04802X^3$	0.97407
11.1° C.	$Y = 90.29 + 2.4913X - 0.74759X^2 + 0.02112X^3$	0.99941
15.6° C.	$Y = 90.29 + 2.3221X - 0.62924X^2 + 0.02935X^3$	0.98142

The above equations fit the data only for that proportion of the population that is not abnormally susceptible or resistant to cold storage, that is, for the

period of storage from one day up to and including the sixth day at 1.0° C., the 11th day at 6.0° C., and the 12th day at 9.0° C. Since at 11.1° C. and above, some development occurs, the storage period was limited by the beginning of adult emergence. Data for 11.1° C., therefore, end at the 15th day, for 15.6° C. at the 8th day, and for 21.0° C. at the 4th day.

Since there is no significant difference between emergence from puparia incubated at 26.7° C. and those stored at 21.0° C. or incubated at 21.0° C. until emergence was complete, for temperatures between 21.0° C. and 26.7°C., the equation for survival becomes $Y = a$ where a equals 92.6. Incubation at 28.0° C. did not reduce emergence. The effects of temperatures higher than 28° C. were not investigated.

Goodness of fit of each equation was tested by calculating the multiple correlation coefficient (R), which is a measure of the correlation between the experimental values of survival and those estimated from the equation. Perfect agreement would result in a value of 1.0000 for R. Since the calculated values of R were close to unity, it was concluded that the equations were good representations of the dependence of survival upon period of storage.

The equations were used to calculate the estimated survival of populations of puparia at different intensities of storage. The points in Fig. 2 represent the observed mean survival calculated from the original experimental data for a large number of different populations.

Using the estimated survival figures, obtained from the equations, a series of curves was drawn to show the dependence of survival upon the temperature for different periods of storage. The equations of these curves were of the form $S = a + b \log T$, where S is the percentage survival, T the temperature of storage (degrees C), and a and b are constants, specific for the period of storage. The equations obtained were as follows:

Period of storage	Equation
1 day	$S = 91.30 + 1.800 \log T$
2 days	$S = 80.00 + 14.780 \log T$
3	$S = 66.85 + 21.988 \log T$
4	$S = 54.00 + 30.200 \log T$
5	$S = 43.59 + 39.358 \log T$
6	$S = 22.72 + 55.640 \log T$
7	$S = -0.16 + 73.269 \log T$
8	$S = 23.87 + 91.226 \log T$
9	$S = 48.26 + 109.436 \log T$
10	$S = 72.14 + 126.940 \log T$
12	$S = 120.00 + 161.000 \log T$
General	$S = a + b \log T$

Plots of the above constants a and b against the time in days showed that they varied with respect to the storage period, according to polynomial regressions similar to those obtained with the original survival period data.

The equations for these regression curves were calculated to be as follows, where X is the storage period in days:

$$a = 93.0788 - 2.00920X - 2.05301X^2 + 0.061187X^3 \quad R = 0.99896$$
$$b = 2.3672 + 1.09518X + 1.68248X^2 - 0.055504X^3 \quad R = 0.99958$$

When the above expressions were substituted for a and b in the general survival temperature equation $S = a + b \log T$, an equation for survival in terms of the two variables, temperature and period of storage, was obtained as follows:

$$S = 93.0788 - 2.00920X - 2.05301X^2 + 0.061187X^3 + \log T$$
$$(2.3672 + 1.09518X + 1.68248X^2 - 0.055504X^3)$$

In Table III, survival, calculated from the above equation, is shown for the experimental temperatures for periods up to 15 days.

TABLE III

CALCULATED VALUES FOR SURVIVAL OF PUPARIA INCUBATED AFTER
COLD STORAGE IN OPEN CONTAINERS

Period of storage (days)	Temperature of storage (° C.)				
	1.0	6.0	9.4	11.1	15.6
1	89.1	92.0	94.0	94.4	95.1
2	81.3	89 8	91.9	92.7	94.3
3	70.2	85.2	89.0	90.4	93.2
4	56.1	79.5	85.4	87.6	92.0
5	39.3	72.3	81.2	84.3	90.6
6	20.3	65.1	76.3	80.5	89.0
7		56.6	70.9	76.2	87.0
8		47.3	64.9	71.4	84.8
9		37.4	58.5	66.3	
10		27.0	51.6	60.7	
11		16.2	44.2	54.7	
12		5.0	36.6	48.3	
13			28.6	41.5	
14			20.4	34.5	
15			11.9	27.1	

Survival of flies from puparia exposed to low temperatures may be illustrated best by reference to the two factors of intensity, temperature, and duration shown on a curved surface. The isometric projection of the surface is shown in Fig. 3.

From these experiments it is clear that survival of housefly puparia in low temperature storage is dependent upon both the temperature and duration of the storage period.   For this species survival decreases as the temperature decreases and as the period of storage increases.
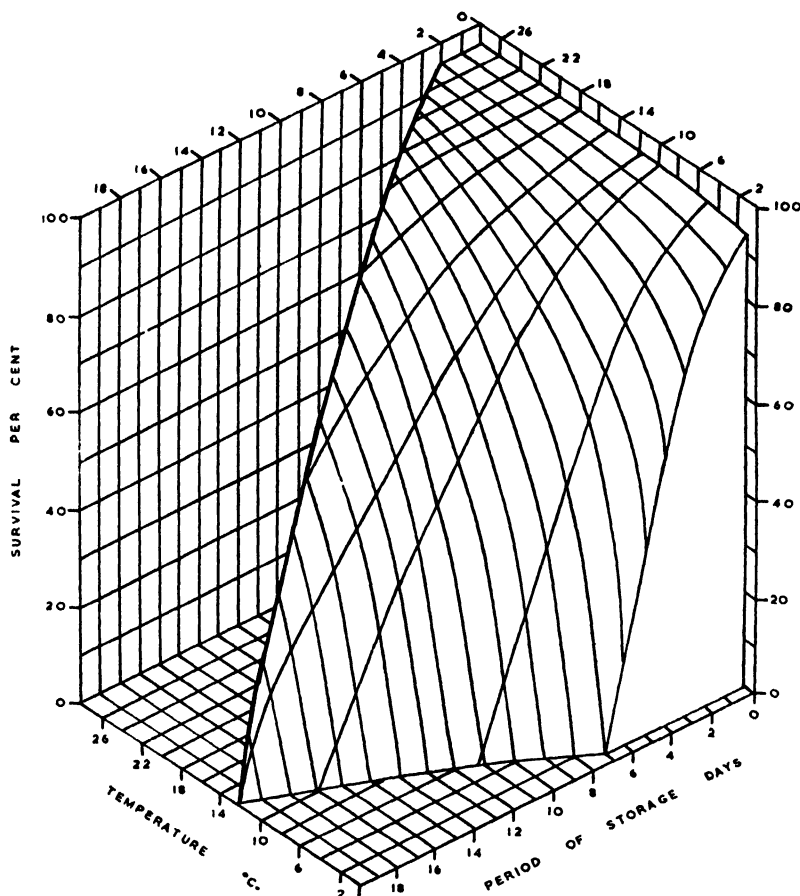
FIG. 3.   *Isometric projection of curved surface representing the survival of puparia exposed to low temperatures.*

The Period of Highest Mortality in Stored Puparia

When puparia are subjected to cold storage only a certain percentage will survive to emerge as adults after being returned to a normal temperature of incubation.   The percentage survival depends upon the intensity of storage. It was assumed at first that cold storage had some detrimental effect that resulted in mortality during the storage period.   Dissection of the unemerged puparia, revealed, however, that death occurred when these were nearly mature, whereas, at the beginning of storage, the puparia were in an early stage of development.   These observations suggested that mortality did not occur during storage but after the puparia had been returned to incubation.

A series of samples of 200 puparia from a single population were, therefore, stored for 10 days at 6.0° C.; a temperature well below the threshold of development. During the storage period and subsequent incubation at 26.7° C., samples were dissected to determine the extent of mortality and development. The results are shown in Table IV. Three arbitrary age classes were set up as follows; each of which is included in the table.

A—Age, 24 to 72 hr. of development.
　　Compound eyes, if formed, still white in color.

B—Age, 72 to 96 hr.
　　Compound eyes amber to light brown in color.

C—Age, 96 to 140 hr.
　　Compound eyes rusty brown to red.
　　Body and appendages well-formed, fuscous.

TABLE IV

Condition of puparia during storage for 10 days at 6.0° C. and
subsequent incubation at 26.7° C.

Time sample dissected	Alive, %			Dead, %			Total No. (100%)
	A	*B*	*C*	*A*	*B*	*C*	
Before storage	80	18	0	2	—	—	200
Immediately after storage	82	18	0	0	—	—	200
After: 24 hr. incubation	30	60	5	5	—	—	200
48	6	38	52	4	—	—	200
72			Emergence begun, no samples				
Emergence complete	0	0	0	5	0	50	200
			(45% emergence)				

About 5% of the population died at an age of less than 72 hr. This portion is probably identical with the 5 to 10% that dies even when no storage treatment is applied. The remainder of the mortality occurred within 40 hr. of emergence when the development was well advanced and the pupae were much like adults in appearance.

Similar results were obtained for storage temperatures of 1.0° C. and 9.4° C.

At temperatures above 1.0° C., therefore, mortality does not normally occur during storage but during subsequent incubation, and at a stage in development when the puparia are nearly mature.

The mechanism by which low temperatures exert a lethal effect upon puparia is not well understood. It can hardly be a simple one, since the

lethal effects are not manifested while puparia are in storage (at least, for periods of storage used in the present experiments) but only after metabolism is resumed and they have reached a certain stage of development.   It appears likely that either the relative rates of various metabolic processes are changed to cause disorganization of the normal sequences of ontogeny at emergence or that some system or its precursor, important to the mature pupa, has been affected.

The Influence of Incubation on Survival of Puparia After Cold Storage

The Incubation Container

When puparia, following storage treatment, were incubated in a standard 10 cm. bacteriological Petri dish complete with cover, it was observed that the proportion that produced adults was higher than expected.   It was also observed that puparia, incubated in 1 X 6 in. open shell vials, had a higher adult emergence than those incubated in open Petri dishes.   Accordingly, several large series of samples of 500 puparia were withdrawn from a population, stored in an identical manner, and incubated in these three different ways.   Adult emergence in covered Petri dishes or in shell vials was significantly higher than in open Petri dishes.   There was no statistically significant difference between adult emergence in closed Petri dishes or shell vials.

Since incubation in a closed Petri dish was more convenient than in an open dish surrounded by a large screen cage to confine the emerging adults, this method of incubation was frequently used.   Incubation in shell vial was used where periodic counts of emergence were required.   The open end of the vial was projected through the wooden end of a small screen cage (6 in. X 4 in. X 2 in.) and held slightly inclined during incubation.   Soon after emergence, adults entered the cage, which could be quickly changed when necessary, the hole being stopped with a cork.   The cages were autoclaved to kill the emerged adults, which were sexed and counted.

During the course of the studies the number of living flies emerging from a large number of puparia was determined.   Incubation was carried on in closed Petri dishes or in 6 in. shell vials.   The survival curves derived from the experimental results are shown in Fig. 4.   Survival is used here to designate the calculated proportion of living puparia as determined from the number of adult flies produced.   It must be 'borne in mind, however, as in former experiments, that those that died during incubation subsequent to cold storage were included in the total number that succumbed.   Thus, the puparia that succumbed between the stage in their development when they were removed from storage and emergence of the adults are not considered as having survived.
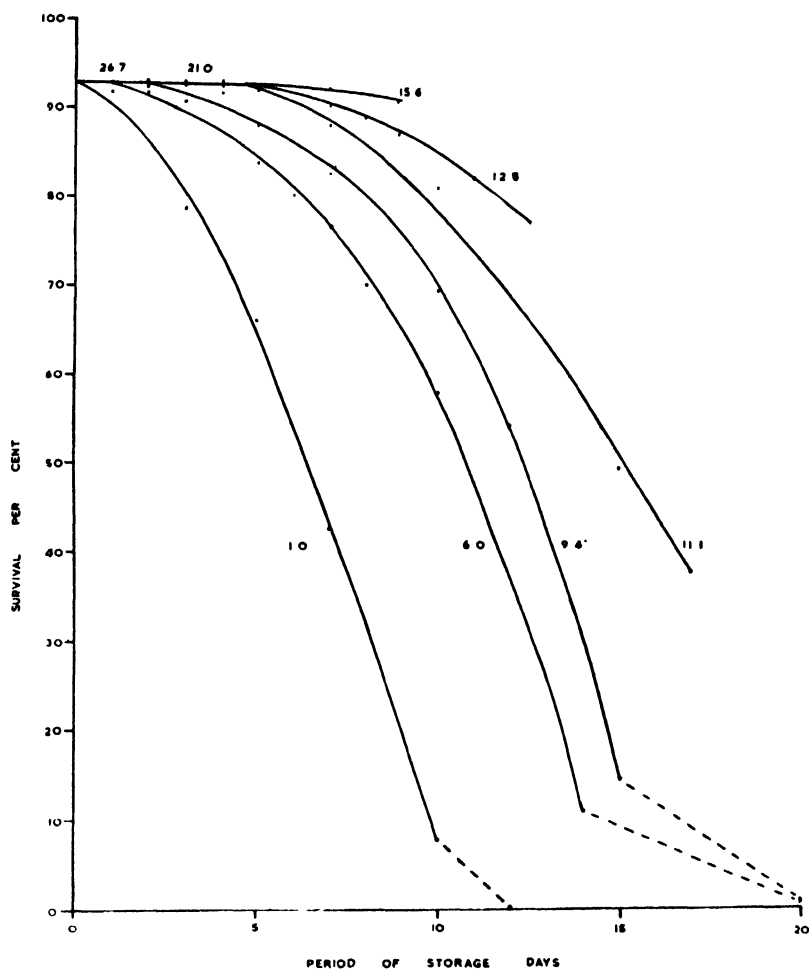
FIG. 4. *Survival of puparia exposed to low temperatures.*

By the use of multiple regression methods, similar to those used for incubation in open dishes, the equations of the survival curves, obtained by plotting survival (Y) against period of storage in days (X), were obtained. These equations are as follows:

Temperature	Equation	R
1.0° C.	$Y = 91.65 - 0.9309X - 1.07706X^2 + 0.03332X^3$	0.9993
6.0° C.	$Y = 94.03 - 1.4397X + 0.01057X^2 - 0.02356X^3$	0.9766
9.4° C.	$Y = 97.52 - 3.0369X + 0.40271X^2 - 0.03780X^3$	0.9863
11.1° C.	$Y = 89.74 + 2.2009X - 0.37626X^2 + 0.00383X^3$	0.9988
12.8° C.	$Y = 92.26 + 0.3059X - 0.04370X^2 - 0.00625X^3$	0.9998
15.6° C.	$Y = 92.60 - 0.0170X + 0.01959X^2 - 0.00416X^3$	0.9999

Since there was no significant difference between emergence from puparia stored at 21.0° C. and those incubated immediately at 26.7° C., survival at temperatures within this range is independent of the temperature and the equation for survival becomes $Y = a = 92.6$.

Using these equations estimated values of survival were calculated for different intensities of storage. The estimated survival figures were used to plot a series of curves showing the variation of survival (S) with temperature (T) for various periods of storage. The equations of these curves are given as follows:

Period of storage	Equation
1 day	$S = 89.70 + 3.730 \log T$
2 days	$S = 85.80 + 6.680 \log T$
3	$S = 80.00 + 11.820 \log T$
4	$S = 72.80 + 17.400 \log T$
5	$S = 64.19 + 25.317 \log T$
6	$S = 54.76 + 32.579 \log T$
7	$S = 43.95 + 41.158 \log T$
8	$S = 32.18 + 50.154 \log T$
9	$S = 19.71 + 59.237 \log T$
10	$S = -7.98 + 82.341 \log T$
12	$S = -63.03 + 125.970 \log T$
General	$S = a + b \log T$

Since the constants a and b varied progressively with the storage period, regressions between a and b and the period of storage (X) were calculated.

$$a = 98.65 - 8.32855X + 0.859359X^2 - 0.107229X^3 \quad R = 0.9991$$
$$b = -4.3641 + 7.38835X - 0.787839X^2 + 0.089650X^3 \quad R = 0.9987$$

Substituting the equations for a and b in the general survival temperature equation $S = a + b \log T$, an equation for survival in terms of both temperature and period of storage was obtained:

$$S = 98.65 - 8.32855X + 0.859359X^2 - 0.107229X^3 + \log T$$
$$(-4.3641 + 7.38835X - 0.787839X^2 + 0.089650X^3)$$

In Table V, survival calculated from the above equation is shown for the experimental temperatures for periods up to 15 days. There was good agreement between the calculated and observed values of survival. An isometric projection of the survival surface is given in Fig. 5.
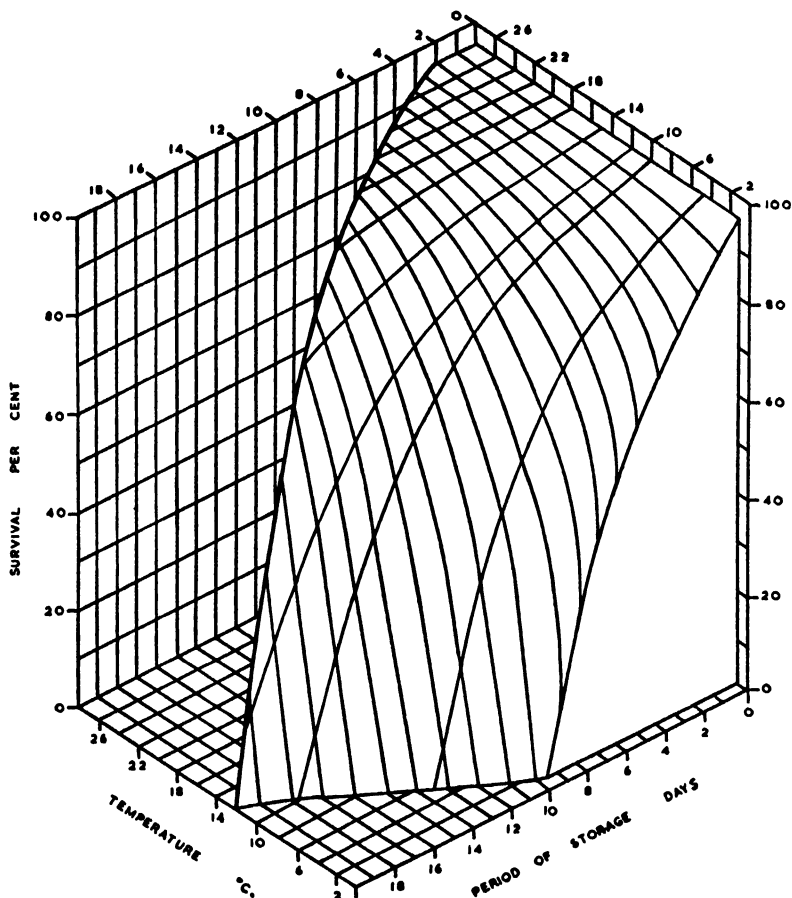
FIG. 5. *Isometric projection of surfaces representing the survival of flies from puparia exposed to low temperatures.*

From a consideration of these results it is clear that when puparia subjected to different intensities of cold storage are incubated in closed Petri dishes or small shell vials survival of the puparia, although differing somewhat from the survival of the puparia as shown in the previous set of experiments, remains as in the former case dependent upon the temperature of storage and duration of the storage period.

A comparison of the two sets of results (Tables III and V) shows that survival was higher when incubation was carried on in closed Petri dishes or in 6-in. shell vials rather than in open dishes with free access to the air. At temperatures from 21.0° to 26.7° C., the type of incubation container did not influence survival. As the intensity of storage was increased, by lowering the temperature or prolonging the exposure, the difference in survival between the two types of incubation increased to a maximum. With further increases in storage intensity the difference in survival became less marked.

TABLE V

CALCULATED VALUES FOR SURVIVAL OF PUPARIA INCUBATED IN CLOSED
PETRI DISHES OR 6-IN. SHELL VIALS

Period of storage (days)	Temperature of storage (° C.)					
	1.0	6.0	9.4	11.1	12.8	15.6
1	91.1	92.9	93.3	93.5	93.6	93.8
2	84.6	90.6	92.1	92.7	93.2	93.9
3	78.5	88.7	91.3	92.2	93.0	94.1
4	72.2	86.5	90.1	91.4	92.5	94.1
5	65.1	83.8	88.5	90.3	91.8	93.8
6	56.5	80.6	86.6	88.8	90.8	93.4
7	45.7	76.4	84.1	87.0	89.4	92.8
8	32.1	71.2	81.0	84.6	87.7	92.0
9	15.1	64.7	77.1	81.7	85.6	91.1
10	--	56.6	72.3	78.1	83.1	
11		46.8	66.5	73.8	80.0	
12		35.0	59.6	68.6		
13		21.0	51.3	62.6		
14		4.6	41.8	55.5		
15		---	30.7	47.4		

It appeared that the superiority of the closed method over the open was related to the gaseous conditions of the atmosphere surrounding the puparia in incubation, probably being a function of differences between the concentration of oxygen, carbon dioxide, or other gaseous metabolites. Since no means of gas analysis were available, this hypothesis was tested partially by experimentation with a variety of incubation containers and population densities, in an attempt to secure variation in the gaseous content of the containers. Population densities between 500 and 10,000 were used. Incubation containers used varied from 6 in. × 2½ in. metal cylinders with screen sides and cardboard cylinders to glass flasks of assorted sizes, either open or partially or completely stoppered. The results are shown in Table VI.

TABLE VI

EMERGENCE OF FLIES FROM PUPARIA STORED AT LOW TEMPERATURES AND INCUBATED IN
DIFFERENT CONTAINERS AND AT VARIOUS POPULATION DENSITIES. (THE PUPARIA
WERE FROM NORMAL POPULATIONS STORED 10 DAYS AT 9.4° C.)

Container	Number of puparia	Emergence of adults, %
Petri dish open	500	51.6 (from Table V)
Petri dish closed	500	72.3 (from Table IX)
Screen and metal can	500 to 10,000	50.5
Metal cylinder, open mouth	5000	55.0
500 cc. Erlenmeyer flask open	500	64.4
	1000	60.7
	2000	55.7
	3000	21.7
	5000	4.7
500 cc. Erlenmeyer flask stoppered	1000	0

In all cases where comparatively free exchange of air was possible, survival was equivalent to that in open Petri dishes. Where diffusion was somewhat more limited, as in cardboard cylinders and metal cylinders with open mouths or open mouth flasks, survival increased though never equalled that of closed Petri dishes. Where diffusion was strictly limited, as in a closed or semi-closed container with a large population, survival was very low.

The Incubation Temperature

The standard temperature used for all incubation was 26.7° C. It has been shown by the storage experiments that incubation between 21.0° C. and 28° C. had no significant effects on puparial survival and observations indicated that extremely high temperatures of incubation invariably decreased survival of the puparia as shown by the reduction in the proportion producing adults. Where populations of 5000 to 10,000 puparia were incubated in thermos bottles, the heat of metabolism accumulated sufficiently to raise the temperature of the puparial mass to 40° C. or more. Under such conditions survival was nil.

Humidity During Incubation

When incubation was performed in closed Petri dishes and shell vials, water of transpiration and the meconial fluid of the emerging adults created damp-ness among the incubating puparia. To test the possibility that this high humidity was the most important factor in the greater survival in these incubation containers, puparia were stored for 10 days at 9.4° C. and incubated in small wooden boxes lined with blotting paper having sliding glass lids. In one series the blotters were kept dry and the relative humidity was 50%. In the other series the blotters were dampened and the puparia mixed with moist sawdust. The relative humidity was 100% and some free surface moisture was present. Survival was slightly better under conditions of high humidity but not sufficiently so as to explain the superiority of closed Petri dish incuba-tion. Survival in incubation at 100% relative humidity was 65%, at 50% relative humidity it was 60%, and in Petri dishes 75%.

Storage Conditions Influencing Survival

Puparia were much less susceptible to changes in physical conditions during storage (except for temperature) than during the incubation period. Thus, the storage container and the gaseous concentration of the microhabitat could be varied within wide limits without affecting survival.

Puparia were stored at low temperatures in open or closed Petri dishes and shell vials in populations of 500, in metal and screen cans in populations as high as 15,000, and in large Petri dishes or tin boxes in numbers up to 50,000 without appreciable influence on survival, so long as sufficient diffusion occurred to maintain gaseous products below a lethal level and supply oxygen. When populations of 5000 to 10,000 were stored in tightly stoppered flasks, they all died, death occurring during the storage period, not in the incubation period.

Humidity was the most important factor during storage. Wide variations from 40 to 80% relative humidity produced no effect on survival. When, however, puparia were stored in moist sawdust, where the relative humidity was 100% and excess superficial moisture was present, mortality was greatly increased and survival was reduced to half the normal value.

The Influence of Larval Rearing on Survival of Puparia During Cold Storage

During the course of the experiments it was found that even when the physical factors of storage and subsequent incubation were constant the results in the case of certain populations of puparia varied considerably from those expected from any given storage intensity. Consequently, some biological factors known to be of importance in rearing were investigated with a view to determining their influence upon survival of puparia in storage.

Although rearing experiments had shown that increased age of the females reduced egg hatchability and survival of the larvae (*vide* first paper in series), in the present study tests on the progeny of three generations of females over a period of 26 days, no correlation was obtained between the age of the mother and resistance of her puparial progeny to cold storage. Similarly, the sex ratio of adults emerging from stored puparia was never significantly different from the sex ratio of unstored puparia. In storage experiments on 10,820 puparia at 6.0° C. for 15 days the sex ratio of the emergents was 50.7% females. Mortality appeared to be alike for both sexes. There was also no correlation between the size of the puparia and survival after cold storage. Small (0.006 gm.) and large (0.025 gm.) puparia produced the same number of adults following exposures to low temperatures.

Small changes in the constituents of the rearing medium such as those due to errors in measurement had no significant effect on survival of puparia after cold storage. If all or part of the alfalfa were replaced by an equal amount of sawdust, however, flies could be reared but the puparia showed a decreased ability to produce adults either when incubated directly or after cold storage. This was shown in a series of tests on 12 populations of flies reared on sawdust medium each of which was paired with a population reared on standard medium as a control. Puparia were subjected to three days' storage at 15.6° C. The differences obtained are shown in Table VII. Statistically they are highly significant.

TABLE VII

Differences in Survival of Puparia Reared on Standard and Sawdust-Containing Media, % ·

Mean emergence from standard medium	93.0
Mean emergence from sawdust medium	81.6
Mean difference	11.4
Number of paired observations	12.0
Value of P	<0.01

At greater intensities of cold storage the susceptibility of sawdust-reared puparia was even more marked. Sawdust-reared puparia stored for 10 days at 6.0° C. gave 10.0% emergence as compared to normal emergence of 56.6% and at 9.4° C., emergence was 28.0% with 72.3% emergence from the controls. It is clear that larvae reared on substandard media produce puparia that are more readily killed by low temperatures.

Under standard conditions of rearing, one battery jar of rearing medium was seeded with 2500 eggs or one small temperature-controlled box with 10,000 eggs. At this density about 72% of the eggs produced puparia. At greater densities (up to 5000 eggs per jar unit of rearing medium) the yield was decreased and the size of the puparia diminished. To determine whether rearing under these conditions altered the susceptibility of puparia to low temperatures during storage, a series of experiments was carried out in which puparia were produced at densities of 2500, 3000, and 3500 eggs per jar unit. These were subjected to five days' storage at 6.0° C. and incubated in closed Petri dishes. Emergence was not significantly different for the three rearing densities. Further trials indicated that, at a density of 5000 eggs per jar unit, adult emergence after storage was still not significantly different from normal.

The influence of larval-rearing temperatures on the resistance of puparia to cold is difficult to determine, since it is almost impossible to rear flies under accurately controlled temperature conditions. Daily changes in the temperature of the medium brought about largely by differences in the rate of fermentation, lateral and vertical gradients, and changes in the temperature preferences of the larvae made it impossible to make correlations between the resistance to storage and the temperature of rearing within the normal temperature range. Under abnormal conditions, however, where the mass of the rearing medium during the larval feeding period remained at 46° C. or more for some time, adult emergence from puparia subjected to low temperatures was noticeably decreased.

In one series of five observations emergence from puparia reared at the normal temperature range was 69.9% following storage at 9.4° C. for 10 days, while emergence from puparia reared where the medium was maintained at 45° to 46° C. for most of the feeding period was 59.8%. The two populations were of the same age when stored. At higher rearing temperatures and greater storage intensities the difference was more marked. Even when puparia were incubated for adult emergence at normal temperatures without storage treatment, a small but significant difference in emergence occurred, when rearing took place at abnormally high temperatures.

In all the experiments it was clear that high temperatures during larval rearing increased the susceptibility of the puparia to death at low temperatures. Although, in some of the experiments it was not possible to attribute

the results solely to the direct effects of rearing temperatures, since the relative age of the puparia would be altered, it would seem that rearing the larvae at low temperatures provided the puparia with greater resistance to the detrimental effects of low temperatures at a later period in life. Whether or not the lower survival of puparia from larvae reared at high temperatures was due to the lack of acclimation, or the possibilities of reducing mortality by slowly lowering the storage temperature, was not included in these studies.

The Relationship Between Low Temperature and Rate of Puparial Development

Housefly puparia stored at temperatures above 11.1° C. produce adults, but below 9.4° C., if exposed for periods at least longer than 24 hr., none emerge. Emergence records indicated that puparia stored at 11.1° C. or higher undergo some development, but that no development occurs during storage at 9.4° C. or at lower temperatures. The threshold of development for puparia, therefore, must be between 9.4° C. and 11.1° C.

In order to define the threshold more precisely and obtain information upon the rate of development above the threshold temperature, 40 duplicate samples of 500 puparia were withdrawn from a single population, placed in shell vials, and stored at various temperatures for periods from 3 to 10 days. They were then returned to an incubation temperature of 26.7° C., the time of return being referred to as zero. Using small screen cages the emergents were counted every eight hours and the sexes recorded. For each sample the mean time for emergence for both males and females was then calculated in hours at 26.7° C. This time was generally two hours shorter for males than for females. The mean of the two times was taken as correctly representing a sample containing equal numbers of males and females. Where two duplicate samples did not differ by more than four hours, the mean of the two was accepted as the best measure of time for emergence for each specific treatment.

After storage at temperatures above 12.7° C., the time for emergence was less than for puparia incubated immediately at 26.7° C., the difference in time representing the amount of development that occurred during the storage period. Time for emergence or amount of development, in all cases, was measured in hours at 26.7° C. Hours of development during storage divided by the days in storage gave the hours of development occurring in 24 hr. at the storage temperature. This method was used to indicate the velocity of development at the different temperatures, the values of which are shown in Table VIII.

Thus it can be seen that the speed of development increases as the temperature increases at the rate shown in Columns 1, 2, and 3. Over this range of temperatures the regression equation was $D = 0 = -4.46 + 0.04885T + 0.03801T^2 + 0.00001T^3$. $R = 0.9929$.

TABLE VIII

AMOUNT OF DEVELOPMENT (MEASURED IN HOURS AT 26.7° C.) OCCURRING DURING 24 HR. AT DIFFERENT STORAGE TEMPERATURES

	Storage temperatures, ° C.								
	26.7	25.0	21.0	19.4	17.0	14.0	12.7	10.0	9.4
Mean amount of development in hours in 40 lots of 500 puparia	24.0 24.0 24.0 24.0 24.0	17.5 20.7 22.2	14.5 15.2 14.1 14.2 14.5 15.0 13.9	10.7 9.5 9.9	7.4 7.6 6.3 7.3 6.6 7.8	2.7 1.9 3.4 3.2 3.3 3.9	2.4 1.9 2.1 2.8 3.2 2.6	0.45 0.00	0.0 0.0
1	24.1	20.7	13.4	10.9	7.4	3.7	2.3	−0.2	−0.6
2	23.8	20.9	14.0	11.2	7.0				
3						3.1	2.4	0.5	0.0

Note.—*1. Calculated from D =   4.46 + 0.04885T + 0.03801T² + 0.00001T³*
2. Calculated from D = −22.31 + 1.7265T
3. Calculated from D = −17.24 + 17.7544 log T
Where D = hours of development. T = temperature ° C.

When the equation is represented as a curved line as in Fig. 6, it is seen that the calculated value of D becomes zero when T is 10.2° C. Thus development ceases at a temperature of 10.2° C. and may be considered as the "threshold of development" of Peairs (14), Shelford (19), and others.
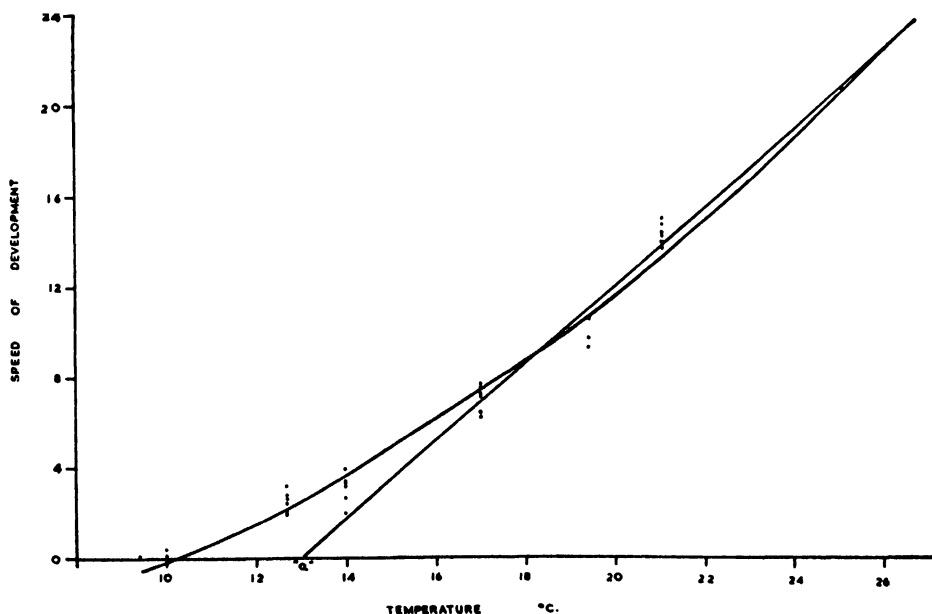
FIG. 6. *The rate of puparial development at temperatures from 0° to 26° C. The units of speed of development is shown in hours.*

Above 17° C. the relationship between speed and temperature may be expressed equally as well by a straight line, the equation of which is $D = -22.31 + 1.7265T$. The equation is represented graphically by the straight line shown in Fig. 6. If the straight line is extrapolated, D becomes zero when T equals 12.9° C., the temperature referred to by Wigglesworth (22) as "zero development" or the "a" point of Shelford (19). By calculation from the linear equation the thermal constant for fly puparia as determined by Peairs' method (14) of *thermal summation* was found to be 80.5 degree-days.

From the observations given above it may be concluded that for housefly puparia the relationship of rate of development to temperature is best expressed by considering velocity as a linear function of temperature above 17° C. and, below 17° C. as a linear function of the logarithm of the temperature. In the present study the upper limits of the biokinetic range were not investigated so that the beginning of departure from linearity, which commonly occurs at high temperatures, is not known. From the time of development calculated from data given by Feldman-Muhsam (8) on the development of housefly puparia at 29° and 34° C., it would appear that the relationship of speed of development and temperature is linear up to at least 34° C.

The Effects of Puparial Storage on the Adults

The storage of puparia at low temperatures not only caused a reduction in adult emergence but also had noticeable effects upon both adult life and the survival of their progeny. This was evident in varying intensities at different storage temperatures.

Puparia from 14 populations were subjected to 10 days' storage at 6.0° C., and then incubated at 26.7° C. Records were kept on oviposition and mortality of the emerging adults and on egg hatching and survival of filial larvae under standard rearing conditions. The results obtained compared with those from the normal breeding populations (p. 29) and with their standard deviations are given in Table IX.

TABLE IX

COMPARISON OF FLIES FROM NONTREATED PUPARIA AND ADULTS
FROM PUPARIA STORED AT 6.0° C.

	Adults from nontreated puparia	Adults from puparia stored 10 days at 6.0° C.
Eggs per female per day (first 10 days of laying)	30 ± 10	5.7 ± 3.1
Percentage of population dead after 10 days of laying	50 ± 10	87.9 ± 7.1
Percentage of eggs that hatched	80 ± 4	60.0 ± 3.0
Percentage of survival of larvae	90 ± 8	52.9 ± 16.0
Percentage survival from eggs to puparia	70 ± 9	34.9 ± 15.1

In the table it can be seen that adult populations, emerging from puparia stored 10 days at 6.0° C., were distinctly abnormal. Adult mortality during the first 10 days of oviposition was high, egg deposition was low, and the egg hatch was low. Larvae, emerging from eggs, did not survive well in rearing while the over-all yield of puparia was only about half that from untreated flies.

At higher storage temperatures the results were somewhat similar and a more detailed study of the effects of puparial storage was, therefore, carried out at 9.4° C. Puparia from a single population were stored at 9.4° C. for different periods from 0 to 10 days. The emergent adults were placed in standard screen cages at population densities of 500 per cage and continuous records obtained similar to those in the previous experiment. The results obtained are given in Table X. The regression equations obtained from the 12 observations are given at the end of the table. In every case the probability levels were well below 0.01 and the regression coefficients were, therefore, highly significant.

TABLE X

EFFECTS OF PUPAL STORAGE AT 9.4° C. ON ADULT LIFE

Cage number	Days in storage	Eggs per female		Percentage of total eggs laid in first 10 days	Hatch, %	Percentage of flies dead in first 10 days			Length of adult life (days)	
		Total	Per day for first 10 days			♂	♀	Population with equal sexes	♂	♀
1	0	526	32.0	60.9	77.0	80.4	31.1	55.7		
2	0	424	24.8	58.5	71.2	91.8	40.3	66.0		
Mean	0	475	28.4	59.7	74.1	86.1	35.7	60.9	10.2	18.2
3	2	356	24.0	67.2	69.2	84.2	43.0	63.6		
4	2	385	25.5	66.2	70.2	86.1	44.0	65.0		
Mean	2	370	24.7	66.7	69.7	85.1	43.5	64.8	10.1	15.6
5	4	321	25.2	78.4	57.9	90.9	47.7	69.3		
6	4	246	17.4	70.9	65.0	94.8	50.6	72.7		
Mean	4	284	21.3	74.6	61.4	92.8	49.1	71.0	9.3	14.6
7	6	321	27.3	85.2	54.3	93.1	65.7	79.4		
8	6	296	24.8	83.9	51.6	93.7	60.3	77.0		
Mean	6	308	26.0	84.5	52.9	93.4	63.0	78.2	8.2	12.1
9	8	179	14.7	81.8	46.4	93.3	58.7	76.0		
10	8	231	20.5	88.7	45.5	94.6	66.5	80.5		
Mean	8	205	17.6	85.7	45.9	94.0	62.6	78.3	8.6	12.3
11	10	127	12.2	96.1	36.8	96.4	82.4	89.4		
12	10	116	11.2	96.1	34.5	96.6	87.2	91.9		
Mean	10	122	11.7	96.1	35.7	96.5	84.8	90.6	7.1	9.3
Regression equations		$Y = 454$ $-32X$	$Y = 28.8$ $-1.43X$	$Y = 60.1$ $+3.54X$	$Y = 76.0$ $-3.89X$	$Y = 85.6$ $+1.13X$	$Y = 33.9$ $+4.52X$	$Y = 59.7$ $+2.83X$	$Y = 10.4$ $-0.30X$	$Y = 17.7$ $-0.81X$

Similar experiments were carried out at 15.6° C. At this temperature male emergents from stored puparia were relatively little affected by storage up to at least eight days but females laid fewer eggs and appeared to have a slightly shorter adult life than normal stock. This is shown in Table XI. Although regression equations were calculated for the number of eggs deposited per female ($Y = 683.4 - 33.6X$) and the length of adult female life ($Y = 18.9 - 0.68X$) only the coefficient for egg deposition was significant.

TABLE XI

EFFECTS OF PUPAL STORAGE AT 15.6° C. ON ADULT LIFE

Cage number	Days in storage	Total eggs per female	Length of adult female life (days)[a]
1	0	797	21.9
2	0	644	16.4
Mean	0	720	19.2
3	2	642	20.1
4	2	522	15.4
Mean	2	582	17.8
5	4	506	15.8
6	4	545	14.9
Mean	4	526	15 4
7	8	430	13.5
8	8	440	14.1
Mean	8	435	13.8

[a] *The records of male longevity are not presented since they were similar to those obtained in the case of nontreated males (vide page 20, Part I of this series).*

From these experiments it is clear that subjecting fly puparia to low temperatures not only caused mortality during the pupal stage but affected the adults that survived the treatment and emerged. Thus, surviving adults had a shorter life and deposited fewer eggs after having passed through a period of cold during the pupal stage. Moreover, the survival of their progeny was noticeably reduced, possibly by a decreased vitality of the larvae. It would appear that in this species the reproductive system and particularly that of the female is especially sensitive to harmful effects of cold temperatures.

Discussion

For many insects the velocity of development is a linear function of the *effective* temperature over a certain portion of the "zone of effective temperatures" (Chapman (7)). The velocity-temperature curve departs from linearity, however, as the minimum and maximum effective temperatures are approached, as shown by Krogh (9). For housefly puparia, between 17° and 34° C., the rate of development is clearly a linear function of temperature but below 17° C. the rate varies with the logarithm of the temperature.

In the present study the effect of low temperatures on the survival of housefly puparia show that between temperatures of 1° C. and 20° C. the mortality of puparia is dependent upon both time and temperature. Equations have been calculated that show that, when temperature remains constant, the mortality varies with time according to equations of the form; $S = a + bX + cX^2 + dX^3$ and that, when time remains constant, the mortality varies with the temperature according to equations of the form $S = a + b \log T$. It has also been shown that the effects of time and temperature can be combined in a single survival equation and that this equation may be represented graphically by a curved surface.

Below 1° C. the effect of temperature on puparia was not investigated. That puparia are very susceptible to temperatures below 0° C. was shown for a small number of samples by Feldman-Muhsam (8). All puparia died after 12 hr. at −3° C., 3 hr. at −7° C., and 1 hr. at −11° C.

Between 20° C. and 28° C., survival of puparia is constant and may be represented by the equation $S = a$, where S is survival and a is a constant having the value 92.6 + 2.4 for the Powell strain, used in the present study.

Above 28° C. the effect of temperature upon puparia was not investigated. Feldman-Muhsam (8), who incubated puparia at 34° C. does not indicate that survival was affected. During the course of the present study it was observed that, when incubation took place in thermos flasks, heat of metabolism was sufficient to raise the temperature to 40° C. or higher. When this occurred, survival was very low.

A number of theories have been presented to explain the killing action of low temperatures. These have been reviewed by Bĕlehrádek (3). In the case of insects that are very resistant to cold, death is apparently the result of ice-formation in the tissues, although some insects can apparently withstand even complete freezing. Resistance to cold, in these insects, is apparently correlated with the prevention of supercooling. In insects that are not very resistant to cold death occurs before freezing of the tissues, provided the time factor is sufficiently long. The mechanism causing death, in this case, must be widely different from that operative during freezing. Bĕlehrádek points out that a number of changes occur in cells when they are chilled and reviews the literature in this connection. Chief among these probably are an increase in viscosity of the protoplasm and abnormalities of mitotic division.

Early theories explaining the mechanism causing death by temperatures above freezing were based on the hypothesis that there was a disproportion between the velocities of several vital functions. Many workers believed that the effect was a result of the accumulation of toxic products that were eliminated at higher temperatures. Bĕlehrádek (2) proposed the hypothesis that an increase of protoplasmic viscosity was responsible for the action of cold. Such an increase in viscosity would hinder molecular movement and affect the rate of biological processes in the organism.

From the results obtained in the present investigations any attempts to explain the mortality of housefly puparia subjected to periods of cold storage must take into account the following experimental observations:

(1) There are variations in resistance to storage between individuals of a population.

(2) Mortality increases as the storage temperature decreases and as the duration of storage increases.

(3) Puparia are not killed by exposure to storage for considerable periods but resume development on being replaced in a favorable temperature, and die only when a definite stage of development has been reached.

(4) The gaseous concentration of the atmosphere during incubation has an influence on mortality following a given storage period, but is a relatively unimportant factor during the storage period.

(5) The age of the puparia, when placed in storage, has a marked effect upon survival.

(6) Storage at temperatures above the threshold of development results in some mortality.

(7) Puparia produced under conditions of high temperature are more susceptible to cold storage.

(8) The individuals surviving a period of cold show evidence of disturbed physiology in the adult stage as evidenced by their shorter life and lowered fecundity.

(9) The reproductive system appears to be affected to a greater extent than other systems, since a reduction of oviposition occurs after storage temperatures that have little effect on longevity. Moreover, the progeny of stored puparia show decreased vitality, there being a reduction of egg hatch and survival of larvae in rearing.

It would appear that an explanation based on the effect of temperature upon the rates of biological processes would best fit these observations.

In studying the effect of temperature on the duration of development in *Drosophila*, Powsner (16) visualized development as the result of a complicated multidimensional network of physical and chemical processes, acted upon both by the genetic make-up and by the environment. He explained the departure from linearity at the extremes of the velocity–temperature curve as follows: "When many reactions proceed simultaneously and only interact at certain points, it is conceivable that all of them will maintain proportionate rates over a long temperature range. Toward both extremes of this range these reactions are likely to become more and more disorganized, so that development becomes abnormally prolonged at these temperatures and both ends of the normal van't Hoff rate curve depressed. . . ."

It would seem that development in the housefly may also be considered the result of a network of interacting processes, all more or less of equal importance. The application of low temperature to this system would probably affect the

rates of some processes more than others, so that the whole system would be disorganized. Beyond a critical point recovery from disorganization does not occur and the result is death.

This may be amplified by further reference to the experimental observations given above.

(1) Individual variations in resistance may be due, either to individual variation in the ability to recover from disorganization or to individual variation in the amount of disorganization brought about by a particular storage treatment. They may be caused by differences in genetic make-up, past environmental experience, or age. The experimental evidence suggests that the last two factors are most important.

(2) If the application of low temperatures causes a disproportion in the rates of ontogenetic development and leads to disorganization, it can be seen that the lower the temperature or the greater the duration of the storage treatment the greater will be the degree of disorganization. More individuals will have passed the critical period of recovery and mortality will therefore increase.

(3) The effect of disorganization of the developmental system need not be apparent immediately. It is more likely that the lethal effect should become manifested towards the end of the pupal period, when the final reactions are poorly synchronized. Since the end of the period is marked by a critical stage in the history of the animal, namely, the emergence of the adult, it is not surprising that death should occur chiefly at this point.

(4) It is more difficult to reconcile the hypothesis with the observation that the gaseous composition of the atmosphere during incubation has an effect on survival. Unpublished data from the Belleville laboratory demonstrate that puparial development in the houseflies can be retarded by placing the puparia in partial vacuum or a medium of high nitrogen concentration. It would seem that when puparia are incubated in shell vials or closed Petri dishes the composition of the atmosphere is such that retardation of some of the developmental processes occurs to partially reverse the temperature effects. During the storage period, when metabolism is low, wide variations in atmospheric composition would not have much influence on survival.

(5) At different periods during the development of pupae, changes in reactions and in the relative rates of reactions can be expected. Bell (4) has shown that the rate of oxygen consumption in pupae of *Galleria mellonella* varied with age, and was at a minimum when pupae were about one-third developed. Similar curves were obtained by Krogh (9) for carbon dioxide production by *Tenebrio molitor* pupae at different constant temperatures. It can be expected, therefore, that the application of low temperature at one period would cause a greater disproportion between rates of reactions than at another period and would consequently result in greater disorganization. In general, greater disorganization of the reactions just previous to emergence would be expected, if the factor causing the disorganization were applied very early in the pupal stage and were cumulative.

(6) Since the rate curve for development of housefly puparia begins to depart from linearity at about 17° C., it would be expected that any temperature below this would disproportionately affect the rates of the various reactions involved. Long storage at temperatures below 17° C. might therefore disturb the network of reactions and result in some mortality, even though development would progress and some emergence occur. It has been shown that some mortality does occur at 15.6° C. Although no experimental data are available between 15.6° and 20° C., it can be seen from the puparial survival shown in Tables V and VIII that little mortality occurs between 17° C. and 20° C. and none above 20° C. where the rate curve is linear.

(7) The temperature at which organisms are reared may influence their ability to withstand cold and it has been suggested that the essential factor is the solidification point of protoplasmic lipoids, which in turn depends on the temperature at which they are formed. It would seem that this is the explanation for the observation that housefly puparia reared at very high temperatures are more susceptible to cold. It may also be that in flies reared at high temperatures the rates of developmental processes are increased and that the processes with increased rates are more susceptible to the disorganizing action of low temperatures.

(8) and (9) If a partial disorganization occurs at the time of adult emergence, it is not surprising that some should be retained in the adult stage and that certain evidences of the disturbance should be apparent in adult life. Disturbed adult physiology in the housefly is shown by a shorter adult life and by impaired fecundity. The reproductive system, particularly that of the female, is apparently more readily disorganized by low temperatures than other parts of the body. Thus oviposition is low after puparial storage. The hatching of the oviposited eggs is also below normal. Whether this is due to decreased fertilization or to factors more closely associated with the female (such as the quantity of yolk) was not determined. At any rate both eggs and the emerging larvae show lowered vitality. By the time pupation has occurred the individuals showing evidence of disturbed physiology have been eliminated and adult emergence from these puparia is normal.

It would appear, therefore, that mortality of housefly puparia, subjected to cold storage, occurs as the result of a disorganization of the interacting network of processes making up development. The disorganization is brought about by a change in the relative rates of the processes of ontogeny at temperatures below 17° C. Its magnitude depends chiefly upon the temperature, the duration of storage and the time (age) when storage is applied. Disorganization in the interaction of developmental processes increases progressively throughout pupal life until, at the time of emergence, the end processes are completely out of synchronization and mortality occurs.

In temperate climates, where the breeding of houseflies is interrupted by winter, considerable interest has developed in the question of how flies pass the winter. The majority of investigators believe that overwintering occurs

in the adult stage. The results of the present study indicate that the over-wintering of flies as puparia is most unlikely, even though the freezing point of puparia was determined by Salt (18) to be about $-12°$ C., or even as low as $-22°$ C. after desiccation. At temperatures below the threshold of development puparia fail to produce adults after 20 to 25 days, while at temperatures above the threshold emergence occurs of at least a portion of the population. When adults emerge from puparia exposed to a period of low temperature, oviposition, egg hatch, and survival of hatched larvae are all so low that the chance of a filial generation surviving is very poor.

Unpublished results from studies at the Belleville laboratory have indicated that both eggs and mature larvae have little resistance to cold. Adults, however, may be kept alive for considerable periods between $10°$ to $15°$ C., although at temperatures much lower they also succumb to cold. It is considered most likely, therefore, that in temperate zones, houseflies pass the winter by a combination of adult hibernation and semicontinuous breeding in favorable situations.

No evidence has been found during this study to suggest that a condition of true diapause occurs during any stage of housefly development.

References

1. BACK, E. A. and COTTON, R. T. Relative resistance of the rice weevil, *Sitophilus oryza* L., and the granary weevil, *S. granarius* L., to high and low temperatures. J. Agr. Research, 28 : 1043-1044. 1924.

2. BĚLEHRÁDEK, J. Le ralentissement des réactions biologiques par le froid est causé par une augmentation de la viscosité du protoplasma. Protoplasma, 3 : 317-326. 1928.

3. BĚLEHRÁDEK, J. Temperature and living matter. (Protoplasma-monographien, Vol. 8.) Verlagsbuchhandlung Gebrüder Borntraeger, Berlin, 1935.

4. BELL, J. The heat production and oxygen consumption of pupae of *Galleria mellonella* at different constant temperatures. Physiol. Zoöl. 13 : 73-81. 1940.

5. BODINE, J. H. Effect of temperature on rate of embryonic development of certain Orthoptera. J. Exptl. Zoöl. 42 : 91-109. 1925.

6. CARTER, W. The effect of low temperatures on *Bruchus obtectus* Say, an insect affecting seed. J. Agr. Research, 31 : 165-182. 1925.

7. CHAPMAN, R. N. Animal ecology. McGraw-Hill Book Company, Inc., New York 1931.

8. FELDMAN-MUHSAM, B. Studies on the ecology of the Levant housefly (*Musca domestica vicina* Macq.). Bull. Entomol. Research, 35 : 53-67. 1944.

9. KROGH, A. On the rate of development and CO_2 production of chrysalides of *Tenebria molitor* at different temperatures. Z. allgem. Physiol. 16 : 178-190. 1914.

10. LARSON, A. O. and SIMMONS, P. Insecticidal effect of cold storage on bean weevils. J. Agr. Research, 27 : 99-105. 1924.

11. LUDWIG, D. Development of cold hardiness in the larva of the Japanese beetle (*Popillia japonica* Newm.). Ecology, 9 : 303-306. 1928.

12. PAYNE, N. M. Freezing and survival of insects at low temperatures. Quart. Rev. 1 : 270-282. 1926.

13. PAYNE, N. M. Two factors of heat energy involved in insect cold hardiness. Ecology, 8 : 194-196. 1927.

14. PEAIRS, L. M. Some phases of the relation of temperature to the development of insects. W. Va. Agr. Expt. Sta. Bull. 208. 1927.

15. PICTET, R. De l'emploi methodique des basses temperatures en biologie. Arch. sci. phys. nat. 30 : 293-314. 1893.

16. Powsner, L. The effects of temperature on the durations of the developmental stages of *Drosophila melanogaster.* Physiol. Zoöl. 8 : 474-520. 1935.

17. Robinson, W. Low temperature and moisture as factors in the ecology of the rice weevil. *Sitophilus oryza* L., and the granary weevil, *Sitophilus granarius* L. Univ. Minn. Agr. Expt. Sta. Tech. Bull. 41. 1926.

18. Salt, R. W. Studies on the freezing process in the insects. Univ. Minn. Agr. Expt. Sta. Tech. Bull. 116. 1936.

19. Shelford, V. E. An experimental investigation of the relations of the codling moth to weather and climate. Ill. Nat. Hist. Survey Bull. 16 : 311-440. 1927.

20. Snedecor, G. W. Statistical methods applied to experiments in agriculture and biology. The Collegiate Press, Inc., of Iowa State College, Ames, Iowa. 1940.

21. Uvarov, B. P. Insects and climate. Trans. Entomol. Soc. London, 79 : 1-247. 1931.

22. Wigglesworth, V. B. The principles of insect physiology. Methuen & Co., Ltd., London. 1939.

STUDIES ON THE HOUSEFLY (*MUSCA DOMESTICA L.*)

III. THE EFFECTS OF AGE, TEMPERATURE, AND LIGHT ON THE FEEDING OF ADULTS[1]

By G. E. Bucher,[2] J. W. MacB. Cameron,[3] and A. Wilkes[4]

Abstract

For a short time immediately after emergence adult houseflies do not consume food. Flies begin feeding at a temperature and age at which they first become active, although to some extent it is dependent on the relative proportions of the sexes, the presence or absence of light, and the accessibility of food. There appears to be no upper age limit at which flies cease to feed. At 27° C. flies died in a very short time if they were not supplied with an abundance of liquid food. All flies died when starved for 24 hr. at 27° C. after having once been fed.

Introduction

For many insect pests a knowledge of their feeding habits has been the only reliable clue to the formulation of satisfactory measures of control. This is true in the case of the housefly and to a certain extent for other insects with somewhat similar habits injurious to orchard and garden crops. Thus the period and rate of the feeding activities, particularly in connection with environmental conditions such as weather has not only an important bearing on the application of control measures but is of practical interest in connection with damage, since in many cases it is due to the quantity and manner in which food is taken by the insect.

Among the saprophagous insects the housefly is probably the best known and it might be assumed that its feeding behavior has been thoroughly studied. This, however, is not the case. Apart from the investigations of Hewitt (4), Graham-Smith (3), Baumberger (1), and others on nutritional requirements and structural makeup of the mouth parts, little has been done on the feeding habits of the adults.

During the present series of studies on the housefly, *Musca domestica* L., general observations indicated the possibility of obtaining more precise information on feeding. In the laboratory rearing of flies it was noted that for some time after emergence adults were inactive and not readily attracted to food. Experiments were, therefore, set up in order to determine the duration of the inactive period under different temperature conditions with a particular view to a determination of the lowest temperature range for feeding and the effects of light and darkness. The purpose of the present paper is to present the results obtained.

Experimental Methods

For the experiments, adults of the Powell strain were used throughout. These were secured from puparia reared in the laboratory and incubated at 26.7° C. In view of the differences in length of life, fecundity, and time of emergence of adults occurring between different populations of flies reared in the laboratory (*vide* Part I of this series*) all puparia used in the present study were withdrawn at random from well-mixed populations. The flies were collected at hourly intervals, placed in small boxes 5½ × 8½ × 1½ in., and supplied with food consisting of the standard milk solution to which had been added a small amount of black India ink. The ink appeared to have no ill effects on the flies. At the end of each experiment the flies were killed in cyanide bottles, sexed, and examined by dissection for signs of feeding. The flies that had fed could be readily detected by the black coloration of the ingested food in the alimentary canal.

Results

In any sample population of flies of the same age there are differences between individual members with regard to the onset of feeding. They do not all begin to feed at the same time. A few begin feeding soon after emergence while others go without food for some time. Thus, at any given time up to about two days after emergence, only a certain percentage of the population has taken food for the first time. The extent to which they feed appears to be dependent largely upon the sex ratio, presence of light, and the temperature of the environment.

Under normal rearing-room conditions a few flies begin to feed soon after emergence from their puparia. Most of them, however, do not begin to feed until about six hours later and it is usually 24 hr. before they have all taken food. At lower temperatures feeding begins much later in adult life. This is shown in Table I. In the table are given feeding records obtained from 23,320 flies tested in environmental temperatures of 27°, 21°, 15°, and 10° C. The flies used in each experiment were taken from populations having equal proportions of males and females.

It may be seen in the table that the percentage of flies that feed increases gradually with age. The increase in rate when plotted against age, as illustrated in Fig. 1 for 27° C., is distinctly sigmoidal in form. At lower temperatures the curves become flattened until at 10° C. they are almost straight lines. Although the data for 15° C. are incomplete, it appears that very few flies begin feeding until they are over eight hours old but by the end of the first day and a half of adult life they had all consumed at least one meal. At 10° C. feeding was very erratic. Some of the flies did not feed for two and one-half days at this temperature. At 6° C. and 0° C. flies did not feed at any age.

Wilkes, A., Bucher, G. E., Cameron, J. W. MacB., and West, A. S. Can. J. Research, D, 26:8-25. 1948.

TABLE I

FEEDING OF FLIES AT DIFFERENT TEMPERATURES

Age (hours from emergence)	Flies feeding, %			
	27° C.	21° C.	15° C.	10° C.
1	1	0		
2	6	1		
3	18	5		
4	32	10		
5	43	17		
6	51	24		
7	58	31		
8	64	36		
9	69	41		
10	73	46	19	
12	80	55	26	
14	86	62	35	
16	91	69	43	
18	94	75	52	
20	96	80	61	
22	97	85	69	
24	98	89	76	31
26	98	93	82	
28	98	96	87	
30	99	98	91	
36	99	99	98	68
60				94
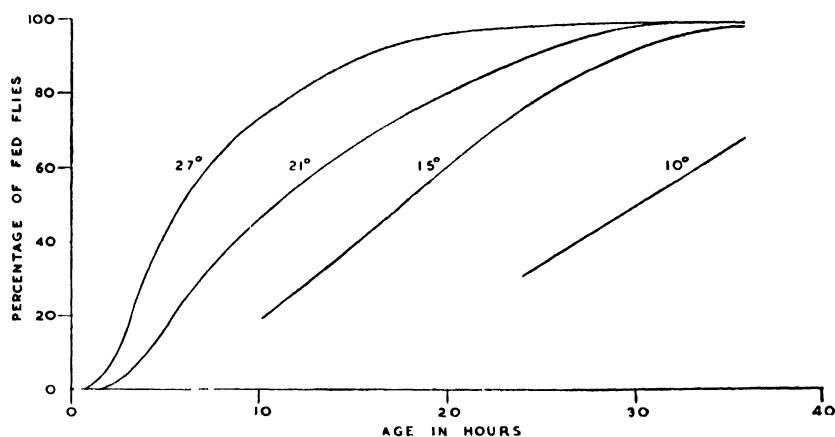
FIG. 1. *The feeding of flies at different environmental temperatures.*

At normal breeding room temperatures (27° C.) male flies begin to feed at a slightly earlier age than females. The difference in time between emergence and the first meal shown by the two sexes becomes greater at lower temperatures. This is shown in Table II. At 15° C. about one-quarter of the males had fed nine hours after emergence whereas less than 1/10th of the females had taken food. At 10° C. after one day, half the males had fed and only one quarter of the females.

TABLE II

DIFFERENCE IN FEEDING OF MALE AND FEMALE FLIES

Age (hours from emergence)	Males				Females			
	No. examined		No. feeding, %		No. examined		No. feeding, %	
	At 27° C.	At 21° C.	At 27° C.	At 21° C.	At 27° C.	At 21° C.	At 27° C.	At 21° C.
1	107	144	0.9	0 0	197	60	1.5	0.0
2	96	99	5.2	1.0	103	48	7.8	0.0
3	113	186	20 4	7 0	153	123	26.8	4.9
4	180	214	38.9	13.1	221	204	26.2	7.4
5	533	483	49 5	20.0	596	436	38.3	12.4
6	227	289	56.4	35 6	176	275	43.8	31.6
7	313	274	63.9	35.4	306	233	55.2	27.5
8	302	388	74.5	40.5	333	396	54.7	35.9
9	218	230	73.9	44 3	193	235	70.5	29.4
12	644	591	84.2	58.0	513	595	78.0	49.9
16		250		70.0		285		61.6
17	320		93.8		340		92.1	
18		372		76.6		382		72.5
19	101		99.0		69		98.6	
20	331	126	96.7	82.5	206	123	95.1	77.2
23	917	558	97.9	88.2	800	367	97.0	87.2
25		271		92.6		159		88.1
26		156		96.8		234		96.6
27	106	111	100.0	95 5	138	100	100.0	90.0
28		197		97 0		215		95.8
30	194		99.0		220		99.1	
36	703	577	99 1	98 9	666	543	97.1	98.0

The absence of light also appears to have a marked bearing on the feeding of houseflies. Experiments carried out in complete darkness and in normally illuminated laboratory rooms show (Table III) that a much smaller percentage of flies will feed in darkness than in the presence of light. Although a few flies, approximately 25%, consume some food in the absence of light, it is probably due to chance contact of the flies with the food dishes in the more heavily populated cages. The results of a series of feeding experiments at temperatures from 0° to 27° C. are shown in Table III.

TABLE III

FEEDING OF FLIES IN LIGHT AND TOTAL DARKNESS. THE FLIES
WERE 36 HR. OLD AT BEGINNING OF THE EXPERIMENT

Temperature (°C.)	Percentage of flies feeding	
	In light	In darkness
27	99.4	27.4
21	99.3	20.6
15	97.9	25.2
10	67.8	7.7
6	0.0	0.0
0	0.0	0.0

Discussion

From the experiments reported here and from observations on numerous populations of flies handled during laboratory propagation it would appear that the feeding behavior of flies is closely associated with general adult activity rather than with anatomical or physiological changes, per se, in the adult. Thus, the temperature limits for feeding are apparently very near the limits for general activity and in this respect *M. domestica* appears to be similar in its feeding habits to grasshoppers. Observations on the feeding of grasshoppers by Parker (5) illustrate this point very clearly.

The temperature at which flies become active and at which they commence feeding is almost the same. At 27° C. about one hour is required after emergence for expansion of the wings and general hardening of the body. At this temperature the adults remain relatively quiescent for about the first four hours. Normal activity is reached in about 15 hr. At 21° C. the hardening process is somewhat slower. Adults remain inactive for from six to eight hours and full activity is reached in about a day. At 15° C. the process is still slower, flies never becoming active as at higher temperatures. Flies are very sluggish at 10° C. and show a much greater tendency to walk than fly. Little movement occurs at 6° C. Flies usually cling to the sides of the cage and only walk slowly when disturbed. At 0° C. there is very little coordinated movement, although the flies cling to the walls of the cage unless they are dislodged by jarring. In complete darkness they behave in much the same way, being very quiescent even at temperatures as high as 27° C.

As in the apple maggot fly, *Rhagoletis pomonella* Walsh (*vide* Caesar and Ross (2)), freshly emerged adults usually apply their mouth parts to the sides and floor of the cage and appear to be lapping up food. For the first hour or so, depending on the temperature, however, little or no food is taken into the body and none appears to enter the alimentary tract until the body and appendages have hardened. After this period the percentage of flies that feed is dependent upon the degree of activity of the population; the greater the activity, the greater the likelihood of the fly coming in contact with the food.

Throughout these studies there appeared to be no upper age limit at which the flies ceased to feed. Flies over 35 days old fed actively at 27° C. At the higher temperature the adults died in a short time if they were not almost constantly supplied with a very liquid diet. Almost all the flies died when starved for 24 hr. at 27° C. and after having once fed they were particularly susceptible to death from starvation.

References

1. Baumberger, J. P. Studies in the longevity of insects. Ann. Ent. Soc. Am. 7 : 323-353. 1914.
2. CAESAR, L. and Ross, W. A. The apple maggot. Ont. Dept. Agr. Bull. 271 : 1-32. 1919.
3. GRAHAM-SMITH, G. S. Flies in relation to disease; non-blood-sucking flies. Cambridge Univ Press, London, 1914.
4. HEWITT, C. G. Houseflies and how they spread disease. Cambridge Univ. Press, London. 1912.
5. PARKER, J. R. Some effects of temperature and moisture upon *Melanoplus mexicanus*, Saussure and *Camnula pellucida*, Scudder (Orthoptera). Montana Agr. Expt. Sta. Bull. 223. 1930.

ACCESSORY TAILS IN FROG TADPOLES, THEIR EXPERIMENTAL PRODUCTION AND SIGNIFICANCE

I. FIVE CASES OF ACCESSORY TAILS IN TADPOLES OF *RANA CLAMITANS* AS A RESULT OF NATURAL INJURY[1]

By David J. McCallion[2]

Abstract

In a collection of several hundred *Rana clamitans* tadpoles, from a pond, five were found having accessory tails. These are described with a probable explanation of their occurrence.

Introduction

Many naturalists have observed and commented on the reproduction of lost tails by lizards. Pliny and Aristotle described some cases of many-tailed lizards. A variety of explanations of the production of accessory tails in lizards were offered by such early investigators as Cardane, Porta, Aldrovandi, Albert-le-Grand, and others. Among them was the belief that double tails were formed from imperfect double eggs. Cuvier discovered that the axial skeleton of a newly regenerated tail was largely cartilaginous, thus providing the clue to a sound explanation of the formation of more than one tail by regeneration. Gachet (6), Müller (9), Fraisse (5), and Tornier (11) produced accessory tails in lizards by experimental methods and contributed to the understanding of their formation. Gräper (7) offered the most satisfactory explanation of this phenomenon.

Accessory tails in urodeles seem to be less well-known. Terni (10) produced a ventral accessory tail in *Triton*. This tail was well-formed, lacking only a caudal artery. One specimen of *Triturus viridescens* having a ventral accessory tail was found and described by Dawson (4). He was able to produce the same result experimentally. Crummy (3) also reported a bifid tail in this species.

There seems to be no report of accessory tails resulting from natural injury in anuran tadpoles. Several investigators (Harrison (8), Barfurth (2), and Avel (1)) have produced accessory tails in these animals in the course of their experiments. Of particular interest in this discussion is the method used by Avel. He cut a hole through the side of the tail of *Rana temporaria* so that a short length of notochord was removed. A new tail grew out from this point, assumed the normal axis, and pushed the old tail aside.

Five Cases of Accessory Tails in Frog Tadpoles as a Result of Natural Injury

In the late fall several hundred tadpoles (*Rana clamitans*) were collected from a pond near McMaster University and taken to the laboratory for use in experiments. Among them were five tadpoles with accessory tails. It was

not possible to photograph them at the time, but the outlines of the tails were drawn and they are described below. These tails were sectioned transversely and examined histologically to determine the extent of regeneration of each of the injured tissues.

<p style="text-align:center">CASE I</p>

On the left side of the tail, about one-third of the distance from the distal end, there was what appeared to be a small, irregular, cutaneous outgrowth (Fig. 1A). At about the same position on the right side of the tail there was a small, nicely-formed tail that had attained a length of about 6 mm. (Fig. 1B). It was directed posteriorly and lay almost in the normal axis. Since this
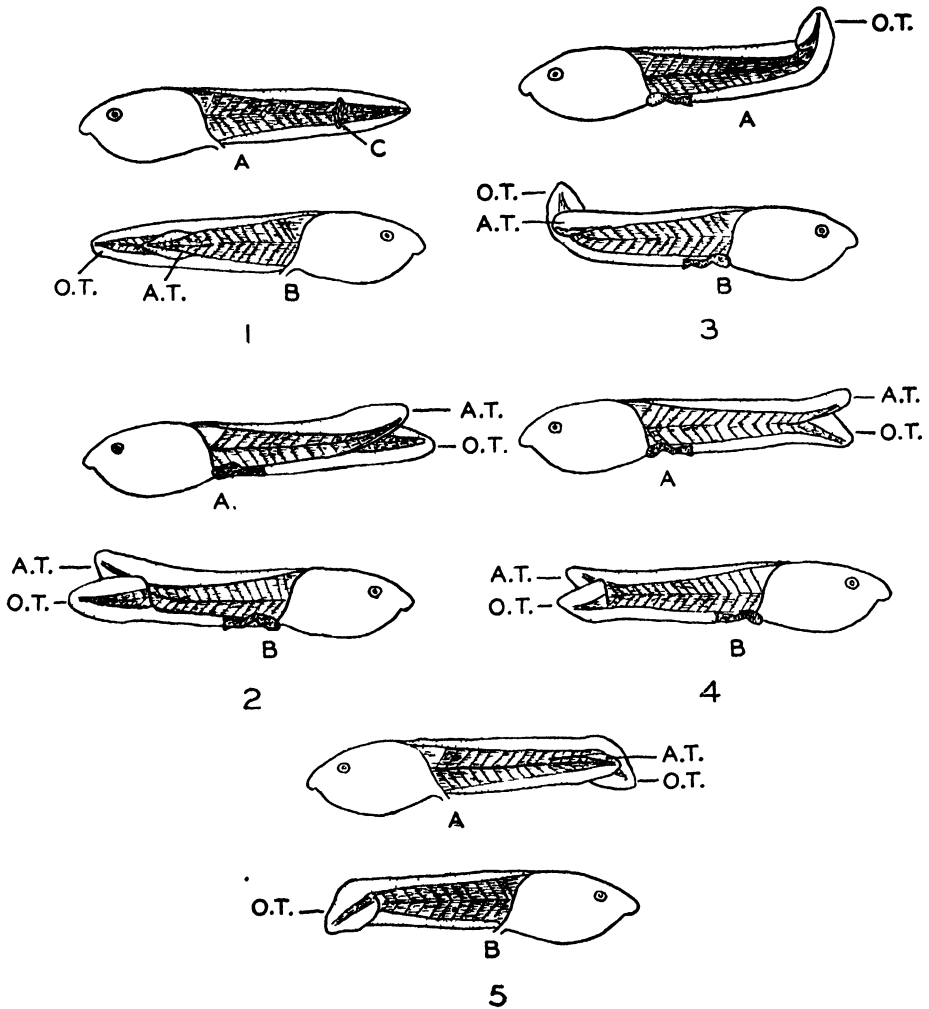
FIGS. 1 TO 5. *Free hand outline drawings to show the nature of accessory tails and old tail ends of tadpoles collected from a pond. Right and left sides of each are shown. Figure numbers correspond to case numbers used in the text. A = Left side; B = Right side; A.T. = Accessory tail; O.T. = Original tail; C. = Cutaneous growth.*

structure so closely resembles those described by Avel (1), it can be assumed
that the tail of this tadpole had been pierced in the same way as in the animals
that he described. Histological examination showed that the small growth
on the left side of the tail was composed almost entirely of epidermis and
mesenchyme. There were a few muscle fibers but no large vessels. In the
tail-like structure on the right side there was a notochord that was a branch
of the original notochord. Mesenchyme and muscle fibers were also present,
but the nerve cord and caudal vessels were absent. The nerve cord of the
old tail was continuous, suggesting that it had not been injured, and therefore,
had had no opportunity for regeneration and could not appear in the
accessory tail.

Cases II, III, and IV

Each of these animals had apparently sustained the same kind of injury,
that is, a deep incision, downward from the dorsal edge of the tail, that severed
the notochord. The resultant accessory tails appeared very much alike (Figs.
2, 3, and 4). In each case the tip of the tail seems to have been incompletely
amputated. As a result, a new tail grew out by regeneration from the poster-
iorly directed surface of the wound while the old tail end remained attached
to the stump by muscle and became consolidated with it at the site of injury.
Thus, any possible regeneration from the anteriorly directed surface of the
wound was mechanically prevented. In Case II the accessory tail was quite
complete, except that the caudal vessels were irregular and there was no
ventral fin fold. Nerve cord, notochord, and muscle were present. The old
tail end, of course, had a normal appearance. The accessory tail and the old
tail end in Case III were not very different from those in Case II. However,
at the site of injury, where both regeneration and healing together of the tissues
had taken place, growth was very irregular. At this point there were three
notochords and four nerve cords. The true notochord and nerve cord of
each tail could be easily identified. The others were simply irregular branches
that were not present at more distal levels. In Case IV the old tail end and
the newly regenerated tail were more completely united at the point of injury
than in the other two cases. In both parts of the tail all of the normal tissues
were present except that the nerve cord had not regenerated into the new tail.
It was probably prevented from doing so by irregular growth of the notochord.
There were two branches of the new notochord at the distal end of the
accessory tail but at more proximal levels this appeared as a large mass. The
nerve cord probably abutted on this and so was prevented from regenerating.

Case V

The tail of this tadpole had suffered the same kind of injury as in the
previous three cases, but the incision had been made from the ventral edge of
the tail upward. The resulting accessory tail (Fig. 5) was similar to those
described above. The incision had been deep enough to sever the nerve cord,
as well as the notochord, for it had regenerated and appeared in the new tail

and also in the old tail end.   Again, the notochord was present in the accessory tail together with some muscle fibers.   The caudal vessels were present and more nearly normal than in any of the other cases.

Discussion and Conclusions

In a subsequent paper the role of the notochord and of the nerve cord in the regeneration of the tail of the frog tadpole will be discussed, particularly with respect to experimentally produced accessory tails.   It has been shown that incomplete amputation of the tails of lizards and urodeles permits the production of accessory tails by regeneration.   Similarly, the occurrence of accessory tails described above demonstrates that, if the tail of the *Rana clamitans* tadpole is incompletely amputated, or injured, in such a manner as to leave the old tail end attached to the stump and also to provide a surface for regeneration of the notochord, an accessory tail results.   Since the structure of the tail is practically identical in the various species of frog larvae it may be assumed that this phenomenon could occur in other species, especially in those that have relatively long aquatic lives.   It is significant that, in each of these five cases, the notochord had been severed, and that in two cases the nerve cord had not been injured.   The nerve cord appeared in the accessory tail only when it had been severed by the injury that made the accessory tail possible.

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References

1. AVEL, M.   L'inhibition de la régénération de la corde dorsale chez les têtards d'amphibiens anoures.   Compt. rend. soc. biol. 109 (5) : 693-695.   1932.
2. BARFURTH, D.   Die experimentelle Herstellung der Cauda Bifida bei Amphibienlarven. Arch. Entwicklungsmech. Organ. 9 : 1-26.   1899.
3. CRUMMY, P. L.   A bifid tail in *Triturus viridescens*.   Proc. Penn. Acad. Sci. 8 : 127-129. 1934.
4. DAWSON, A. B.   A ventral accessory tail in *Triturus viridescens* and its duplication experimentally.   Anat. Rec. 52 (2) : 139-145.   1932.
5. FRAISSE, P.   Die Regeneration von Geweben und Diganen bei den Wierbelthieren besonders bei Amphibien und Reptilien. Kassel und Berlin.   1885.
6. GACHET, H.   Mémoire sur la 1éproduction de la queue des reptiles sauriens.   Actes Soc. Linnéene Bordeax, No. 36.   1834.
7. GRÄPER, L.   Über eine dreischwanzige Eidechse mit sieben Schwanzskeleten.   Arch. Entwicklungsmech. Organ. 27 : 640-652.   1909.
8. HARRISON, R. G.   The growth and regeneration of the tail of the frog larva.   Arch. Entwicklungsmech. Organ. 7 : 430-481.   1898.
9. MÜLLER, H.   Über Regeneration der Wirbelsäule und des Rückenmarkes bei Tritonen und Eidechsen.   Abhandl. Senck. naturforsch. Ges., Bd. V.   1864–65.
10. TERNI, T.   Studio anatomico di code doppie sperimentalmente ottenute nei Tritoni. Arch. Zool. Ital. 16 (½) : 412-417.   1931.
11. TORNIER, G.   Über experimentelle erzeugte dreischwanzige Eidechsen und Doppelglied- massen an Malchen.   Zool. Anz., Bd. XX.   1897.

REDUCTION OF *TRIAENOPHORUS* INFESTATION IN WHITEFISH BY DEPLETION OF THE CISCO POPULATION[1]

By RICHARD B. MILLER[2]

Abstract

In 1940 the catch of cisco in Lesser Slave Lake increased to over a million and a half pounds; it has remained at this high level for eight years, in the last two of which the catch has exceeded three million pounds. The age composition of the catch has decreased from nearly 80% six-year-olds or older to nearly 80% two-year-olds. During the period 1944-1947 the number of *Triaenophorus* cysts in the whitefish has decreased from 265 per 100 fish (102.7 per 100 lb.) to 26 per 100 fish (6.8 per 100 lb.). This paper presents the evidence that indicates that this reduction of infestation in the whitefish has been a result of the depleted cisco population.

Introduction

The tapeworm, *Triaenophorus crassus* Forel, lives as an adult in the intestine of the northern pike, *Esox lucius* L. The first larval stage (procercoid) is passed in the copepod, *Cyclops bicuspidatus* Claus, and the second larval stage (plerocercoid) is passed as a cyst in the flesh of, principally, the coregonine fishes. The presence of these cysts in the flesh of whitefish seriously interferes with the marketability of the fish; some means of reducing this infestation is highly desirable.

The study of the details of the parasite's life history was begun in Alberta lakes in 1939 in the hope that their complete elucidation would point the way to control measures. This study of the life history details has been more or less completed and the results published (2, 3, 5). The various possibilities of reducing or eliminating *Triaenophorus* infestation, made apparent by a knowledge of the life history, have been outlined in an Alberta Government publication (4).

One of the methods suggested in this publication (4), an attack on the early free-swimming larval stage, has been attempted (Miller and Watkins, 7); in this experiment an unsuccessful attempt was made to acidify a lake in order to kill the coracidia of the parasite. Further experiments on this possibility of control are still being conducted. The present paper reports

the results of another, long-term method of attack. In this method the reduction of infestation is sought through depletion of the tullibee* (cisco) population in Lesser Slave Lake.

The publication (4), referred to above, outlined the main arguments for regarding the tullibee as the principal second intermediate host. These arguments are here recapitulated:—

1. In any lake where infestation of whitefish and tullibee occurs, the tullibee are always the more heavily infested.

2. In at least six Alberta lakes there are whitefish but no tullibee; northern pike and *Cyclops bicuspidatus* are present. In all of these lakes the whitefish are free of *Triaenophorus* infestation. It would appear that the tullibee are necessary in the life cycle of the parasite.

3. Miller (6) has shown that parasitized whitefish grow more slowly than clean whitefish, whereas parasitized tullibee grow at a rate not significantly slower than clean tullibee. The association of host and parasite that produces the lesser disturbance is surely the more natural.

These three pieces of evidence suggest strongly that if the tullibee could be eliminated from infested lakes the whitefish would become free of infestation. Complete elimination of a fish in a large lake is practically an impossibility. That complete elimination of tullibee to accomplish parasite reduction is not necessary may be seen by examining the data in Table III. This table shows the degree of infestation of each age group of tullibee in Lesser Slave Lake; it is apparent that tullibee do not become heavily infested until they are four-year-olds. To effect an appreciable reduction in parasites, then, it is only necessary to remove tullibee four years old and older. Accordingly, in 1940, the Provincial Fisheries Branch, faced with a large demand for mink feed and understanding the chance of parasite reduction, removed virtually all limitations to the taking of tullibee in Lesser Slave Lake.

The Depletion of the Tullibee

For the five year period ending in 1939 the average annual catch of tullibee in Lesser Slave Lake was 63,000 lb. Catches since then are shown in Table I.

TABLE I

THE ANNUAL* CATCH OF TULLIBEE IN LESSER SLAVE
LAKE SINCE 1940 (LB.)

1940-41 – 1,504,900	1944-45 – 1,349,020
1941-42 – 1,993,900	1945-46 – 1,491,981
1942-43 – 1,770,600	1946-47 – 3,556,587
1943-44 – 1,475,900	1947-48 – Over four million

* *The fishing year does not correspond to the calendar year but comprises the winter and succeeding summer.*

* *The ciscoes in western Canada are usually referred to as tullibee, although species other than tullibee, sensu strictu, commonly occur.*

The enormously increased catches of the past eight years and particularly of the last two years have had a profound effect on the tullibee population. This effect may be traced in the changing age composition of the catches as shown in samples taken since 1941. There has been no change in the commercial gear used to catch the tullibee throughout this period. The gear used is gill net of $2\frac{3}{4}$ in. mesh, stretched measure. All samples were taken with this gear. The age composition of these samples is shown in Table II. Unfortunately, no data are available for 1946. Altogether the ages of 1348 tullibee have been determined.

<div align="center">TABLE II</div>

<div align="center">THE AGE COMPOSITION OF THE SAMPLE CATCHES OF TULLIBEE
FROM LESSER SLAVE LAKE, 1941-1947</div>

Year	Number of fish in sample	Ages and percentage of each age in sample								
		1	2	3	4	5	6	7	8	9
1941	118	0	0	2.6	5.8	12.7	42.3	26.3	10.2	0
1942	85	0	0	4.7	1 2	16.5	65.9	11.7	0	0
1943	99	0	0	6	9	27	38	17	2	0
1944	545	2.3	5 8	4.4	8.8	17.4	27	27.5	6.4	0.4
1945	100	0	18	7	12	16	17	24	5	1
1947	401	4.7	78 5	15	1	0.7	0.2	0	0	0

At the time when heavy fishing began in Lesser Slave Lake the catch consisted of about 80% six-year-old or older fish. Slightly more young fish showed up in the samples of 1942. By 1943 about half the catch (as represented by the sample) was less than six years old; in 1944 and 1945 one- and two-year-old fish appeared in the samples. In 1947, 78.5% of the sample was of two-year-old fish and only 16.9% was older than two.

These figures suggest a great reduction in the tullibee population. The effect that this has had on the quantity of *Triaenophorus* cysts in the tullibee is discussed in the next section.

The Change in the Infestation of the Tullibee

At the beginning of the period of increased tullibee catches close to 100% of these fish were infested with cysts of *Triaenophorus*. More or less casual checks from time to time showed no appreciable change in the next few years. In 1944 a thorough 'measurement of the infestation of each age group of tullibee was made; further careful examinations were made in 1945 and 1947. The results of these are shown in Table III.

With the removal of the older tullibee through heavy fishing there has been a spectacular reduction in total percentage infestation from close to 100 to about 11%; the average infestation has decreased from 890 cysts per 100 fish (all ages) to 15.4.

TABLE III

THE INFESTATION OF TULLIBEE OF LESSER SLAVE LAKE WITH CYSTS OF *Triaenophorus crassus*, 1944-1947. THE FIGURES IN THE TABLE REPRESENT CYSTS PER HUNDRED FISH

| Date | Number of fish | Age of fish | | | | | | | | | Av. all ages | Fish infested, % |
		1	2	3	4	5	6	7	8	9		
Jan.-Feb. 1944	332	—	—	82	760	1078	910	1012	437	—	890	93
May-Oct. 1944	213	0	15.6	200	280	992	1225	820	1121	633	754	76
Jan.-Feb. 1945	100		5.6	28 5	733	770	880	835	540	100	591	75
Oct. 1947	100	0	8.4	20	—	800	1200	—	—	—	37	10
Nov. 1947	201	0	6 6	40 9	0	300	—	—	--	-	15.4	11.4

Carlander (1), studying tullibee in Lake of the Woods, Minn., mentions a decrease in infestation with *Triaenophorus*. He also finds a shift to the younger age groups of tullibee associated with heavier fishing and it is possible that the parasite reduction is related to this change.

If the argument given in the introduction is correct, i.e., if the tullibee is the principal second intermediate host of *Triaenophorus crassus*, then the whitefish in Lesser Slave Lake should show decreased infestation. In the following section data on whitefish infestation are presented.

Triaenophorus Cysts in Whitefish

The first investigation of these cysts in whitefish of Lesser Slave Lake was made in the summer of 1940 when 123 whitefish were cut up in a search for the parasites. Thirty-five per cent of these fish were infested at a rate of 52.5 cysts per 100 lb. and 131 cysts per 100 fish. In 1941 a sample of 81 whitefish was found to be 24.7% infested. No cyst count was made. Fishermen reported similar high infestations during the seasons of 1942 and 1943.

Large samples of whitefish were carefully cut up to make cysts counts in 1944, 1945, and 1947. Scales were taken from each whitefish and from these the ages of the fish have been determined. The data are shown in Table IV.

There has been a very considerable reduction of infestation in the whitefish. The average of cysts per 100 fish has decreased from 265 to 26, the average of cysts per 100 lb. from 102.7 to 10.5, and the percentage of fish infested from 30.8 to 6.8. That this reduction is not just random fluctuation is shown by the figures for cysts per 100 fish of each age group. Significant numbers of fish were examined in the three- to seven-year-old age groups. It can scarcely be chance that there is a consistent reduction of infestation in each of these age groups from 1944-1947.

TABLE IV

THE INFESTATION OF WHITEFISH OF LESSER SLAVE LAKE WITH CYSTS OF *Triaenophorus crassus*, 1944-47.
FIGURES IN PARENTHESES GIVE NUMBER OF FISH EXAMINED

Date	Number of fish*	Age and cysts per 100 fish							Av. cysts per 100 fish	Av. cysts per 100 lb.	Fish infested, %
		3	4	5	6	7	8	9			
Sept. 1944	490	240 (51)	65 5 (203)	94 3 (107)	260 (39)	882 (34)	1556 (38)	453 (8)	265	102.7	30 8
Oct. 1945	500	0 (2)	94 (104)	63 (186)	189 (121)	408 (60)	1054 (22)	40 (2)	185	54.7	17.4
Oct. and Nov. 1947	601	0 7 (139)	6 5 (355)	41 (49)	35 (23)	328 (14)	345 (9)	357 (7)	26	10 5	6 8

* The total number of fish examined does not agree exactly with the total of the figures in parentheses as a few fish older than nine and younger than three have been omitted.

Discussion and Conclusions

The data presented in this paper show that when the tullibee population consists largely of young fish the infestation with *Triaenophorus* cysts becomes very small. The data also show that, since the tullibee is the principal second intermediate host of the parasite, the small parasite population in tullibee results in a small parasite population in the whitefish as well. The record of the age composition of tullibee populations in Lesser Slave Lake since 1941 suggests that the present preponderance of young fish has resulted from overfishing. It follows, therefore, that overfishing of the tullibee will produce a decreased infestation of whitefish with *Triaenophorus* cysts. The author feels that the evidence for this conclusion is strong enough to warrant advising administrators who have a *Triaenophorus* problem to try overfishing of tullibee as a cure. The tullibee gear may take some of the young of other species of fish but the damage done in this way is not commensurate with the achievement of significantly reduced infestation.

Acknowledgments

References

1. CARLANDER, K. D. Growth, length-weight relationship and population fluctuations of the tullibee, *Leucichthys artedi tullibee* (Richardson), with reference to the commercial fisheries, Lake of the Woods, Minnesota. Trans. Am. Fisheries Soc. 73 : 125-136. 1945.

2. MILLER, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. I. Introduction and the life of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas) in the definitive host, *Esox lucius*. Can. J. Research, D, 21 : 160-170. 1943.

3. MILLER, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. II. The eggs, coracidia, and life in the first intermediate host of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas). Can. J. Research, D, 21 : 284-291. 1943.

4. MILLER, R. B. Suggestions for experiments in the control of the pike-whitefish tapeworm, *Triaenophorus crassus*. Dept. Lands Mines, Fisheries Branch, Alta. 1944.

5. MILLER, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. IV. The life of *Triaenophorus crassus* Forel in the second intermediate host. Can. J. Research, D, 23 : 105-115. 1945.

6. MILLER, R. B. Effect of *Triaenophorus* on growth of two fishes. J. Fisheries Research Board Can. 6 (4) : 334-337. 1945.

7. MILLER, R. B. and WATKINS, H. B. An experiment in the control of the cestode, *Triaenophorus crassus* Forel. Can. J. Research, D, 24 : 175-179. 1946.

OBSERVATIONS ON *LEUCOCYTOZOON* INFECTIONS IN BIRDS RECEIVING PALUDRINE, ATEBRIN, AND SULPHAMERAZINE[1]

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Abstract

Paludrine, atebrin, and sulphamerazine neither prevented nor cured infections with *Leucocytozoon simondi* in ducklings, nor did they have any noticeable effect on the course of the infections. Paludrine failed to cure an established infection of *Leucocytozoon sakharoffi* in a crow. The extensive 'tissue stages' of these parasites probably explain the negative results.

The extensive tests on the use of atebrin and paludrine for the prevention and cure of malaria (Fairley (5, 6), Curd, Davey, and Rose (2-4)) have supplied further data on the biology of the parasites that cause this disease. It was considered of interest therefore to test the effects of these drugs on the parasites in the related genus *Leucocytozoon*.

O'Roke (8) tested the efficacy of quinine sulphate and dihydrochloride and Plasmochin on the gametocytes of *Leucocytozoon simondi* in ducks but found them ineffective. Coatney and West (1) administered atebrin to a great horned owl and a juvenile red-tailed hawk that had heavy infections of a spindle type of *Leucocytozoon*. They concluded that the drug has some effect inasmuch as it produced morphological changes in the form of clear vacuoles in the parasites and the host cells.

In the present experiments the prophylactic and curative value of paludrine and atebrin on *L. simondi* was tested as follows: 19 10-day-old ducklings were left in the open to be bitten by black flies. Paludrine and atebrin were given orally to eight of the ducklings 12 hr. after the first exposure to black flies and daily thereafter for 15 days. Four of these ducklings received 1 mgm. paludrine per day and four received 2 mgm. atebrin. The other ducks served as controls and a number in this group showed infection in the blood 11 to 12 days after their first exposure to black flies. Three of these ducklings were each given 2 mgm. atebrin t.i.d. for four days following the appearance of infection. Four of the ducklings received 1 mgm. paludrine t.i.d. for four days following the appearance of parasites in the blood. This left four birds in the entire group as controls that received no treatment. Blood smears, which were made daily from each bird, were stained with Giemsa and examined for parasites. The date on which the first parasites were observed in the blood as well as the number in a count of one minute on the smear were noted (Table I).

It will be observed from the data in this table that neither paludrine nor atebrin in the above dosages prevented or cured infection with *L. simondi*

TABLE I

TREATMENT WITH PALUDRINE AND ATEBRIN

Duck No.	Treatment	Date gametocytes observed	Date of peak infection	Number parasites in 1 min. count on smear at peak of infection
10	1 Mgm.	June 28	July 9	38
11	paludrine	June 24	June 28	37
12	daily,	June 28	June 30	24
13	June 13 to 28	June 25	June 29	13
14	2 Mgm.	June 24	June 25	31 Died July 9
15	atebrin	June 24	June 25	130 Died June 30
16	daily,	June 24	June 28	91 Died July 12
17	June 13 to 28	July 4	July 5	6
18		June 23	—	61 Died June 23
19	Controls	June 24	June 28	147
20		June 25	June 26	36
21		June 29	June 30	56
22	2 Mgm. atebrin	June 25	June 30	39
23	t.i.d.,	June 25	June 27	146
24	June 25 to 29	June 24	June 26	82
25	1 Mgm.	June 25	June 28	34
26	paludrine t.i.d.,	June 24	June 29	114 Died July 10
27	June 25 to 29	June 25	June 29	74
28		June 24	June 29	91

in ducks. There was no marked difference in the level of infection in the blood in the treated vs. the control birds. No morphological changes were noted in the parasites that might have been caused by the drugs. Fairley (6) found that although paludrine did not destroy gametocytes of *Plasmodium* in man it prevented their further development in mosquitoes that had fed on gametocyte carriers receiving the drug. No such effect was observed in the present study as the parasites developed to oökinetes in the black flies that fed on a bird receiving the drug.

In a second experiment three ducklings that were two weeks old were each given orally 50 mgm. sulphamerazine daily for over three weeks, beginning on the date on which they were first exposed to black flies. Two ducklings of the same age were kept as controls. Some of the results of this experiment are shown in Table II. It will be observed that the drug did not prevent infection becoming established nor did it have any apparent effect on the level of infection reached in the treated vs. the control birds.

A single crow, showing a high gametocyte level of *L. sakharoffi* and evidence of leg paralysis thought to be due to the infection, was given 2 mgm. paludrine four times a day for four days but it failed to recover.

TABLE II

TREATMENT WITH SULPHAMERAZINE

Duck No.	Treatment	Date gametocytes observed	Date of peak infection	Number of parasites in 1 min. count on smear at peak of infection
33	50 Mgm.	July 16	July 26	23
34	sulphamerazine,	July 13	July 18	44
35	July 4 to 31	July 16	July 19	57
36	Controls	July 16	July 20	41
37		July 12	July 19	151

Discussion

It appears from these experiments that the drugs paludrine, atebrin, and sulphamerazine, in the dosages used, have no value in preventing infection with *Leucocytozoon simondi*. Neither did the two former drugs cure infections that were established. The dosages of paludrine and atebrin on a weight basis are slightly greater than those that are used successfully on man. The results with atebrin are perhaps not comparable with those of Coatney and West (1) who administered large quantities of the drug. The sulphamerazine dosage was similar to that which has been used successfully by Swales (9) for coccidia in chickens. It may be argued that the drugs are ineffective against the sexual forms of the parasite. However, these must arise from the stages in the tissues. Unless the 'tissue stages' that produce gametocytes do so exclusively, and have a long life, this is not the sole explanation as otherwise the infections would not persist in the birds without more or less continuous asexual development. It seems more probable that the drugs were not effective against the 'tissue stages' (Huff (7), Wingstrand (10)) rather than that the dosages were inadequate. Such an explanation would support the view that the preventive and curative effects of these drugs on malaria are related to the nature and extent of the 'tissue stages'. It will be of interest, for these reasons, to explore more fully the relationships between schizogony and gametogony in *Leucocytozoon* and to obtain a more complete understanding of the sequence of asexual stages.

Acknowledgments

References

1. COATNEY, G. R. and WEST, E. Some notes on the effect of atebrine on the gametocytes of the genus *Leucocytozoon*. J. Parasitol, 23 : 227-228. 1937.
2. CURD, F. H. S., DAVEY, D. G., and ROSE, F. L. Studies on synthetic antimalarial drugs. I. Biological methods. Ann. Trop. Med. 39 : 139-156. 1945.

3. CURD, F. H. S., DAVEY, D. G., and ROSE, F. L.   Studies on synthetic antimalarial drugs. II. General chemical considerations.   Ann. Trop. Med. 39 : 157-164.   1945.

4. CURD, F. H. S., DAVEY, D. G., and ROSE, F. L.   Studies on synthetic antimalarial drugs. X. Some biguanide derivations as new types of antimalarial substances with both therapeutic and causal prophylactic activity.   Ann. Trop. Med. 39 : 208-216.   1945.

5. FAIRLEY, N. H.   Chemotherapeutic suppression and prophylaxis in malaria.   Trans. Roy. Soc. Trop. Med. Hyg. 38 : 311-365.   1945.

6. FAIRLEY, N. H.   Researches on paludrine (M-4888) in malaria.   Trans. Roy. Soc. Trop. Med. Hyg. 40 : 105-162.   1946.

7. HUFF, C. G.   Schizogony and gametocyte development in *Leucocytozoon simondi,* and comparisons with *Plasmodium* and *Haemoproteus.*   J. Infectious Diseases, 71 : 18-32. 1942.

8. O'ROKE, E. C.   A malaria-like disease of ducks caused by *Leucocytozoon anatis* Wickware. Univ. Mich. School of Forestry Conserv. Bull. 4, pp. 44.   1934.

9. SWALES, W. E.   New methods of controlling caecal coccidiosis in chicks.   Can. J. Comp. Med. Vet. Sci. 11 : 5-10.   1947.

10. WINGSTRAND, K. G.   On some haematozoa of Swedish birds with remarks on the schizogony of *Leucocytozoon sakharoffi.*   Kgl. Svenska Vetenskapsakad.   Handl. 24 : 1-31.   1947.

ON THE CHEMOTHERAPY OF CAECAL COCCIDIOSIS
(*Eimeria tenella*) OF CHICKENS

VI. A NOTE ON THE METABOLISM OF CAECAL EPITHELIUM, NORMAL AND PARASITIZED[1]

By H. B. COLLIER[2] AND W. E. SWALES[3]

Abstract

The respiration and anaerobic glycolysis of caecal tissue of chickens were measured; the values obtained were similar to the corresponding values for rat intestine.

Infection of the caecal mucosa with coccidia (*Eimeria tenella*) produced no significant change in metabolic rate. Sulphamerazine, which is coccidiostatic *in vivo*, did not affect the metabolism of tissue strips.

Introduction

Work on the control of caecal coccidiosis of chicks has demonstrated that the sulphapyrimidine drugs are strongly coccidiostatic and that they exert their strongest inhibitory effect upon the merozoites (Swales (12), Ripsom and Herrick (10)). Recently it has been shown that the schizonts containing second generation merozoites are largely destroyed by sulphamethazine (Farr and Wehr (5)). Knowledge of the relative metabolic activity of the normal host tissues and of the tissues at various stages in the parasite's life cycle would be of great value in work designed to clarify the mode of action of coccidiostatic drugs; it might also lead to a means of testing chemicals *in vitro* for coccidiostatic properties. The work herein reported was an attempt to find differences in metabolic activity between normal caecum and caecal tissue parasitized by various stages of *Eimeria tenella*.

Christophers and Fulton (1) initiated the biochemical study of blood in which malaria parasites, *Plasmodium* spp., were established. More recent studies of malarial blood have been carried out by McKee *et al.* (9), Evans (4), and Hellerman *et al.* (6). They were able to demonstrate an increased metabolic activity in parasitized erythrocytes, and under some conditions the metabolism of the parasites was inhibited by antimalarial drugs such as quinine and quinacrine.

Experimental

Methods

Chicks of various breeds, from 7 to 10 weeks of age, were used. To obtain normal tissue the chicks were taken from batteries in a room kept free of coccidia, killed by decapitation, and the caeca were opened, rinsed in isotonic

saline at 40° C., and rapidly prepared as described below. Parasitized caeca were similarly obtained from chicks from the same group that had been dosed per os with 100,000 sporulated oocysts of *E. tenella* and killed at appropriate times thereafter.

The metabolic activity of caecal tissue was measured by methods similar to those employed in a study of tissues of the mammalian alimentary tract by Dickens and Weil-Malherbe (2) and by Lutwak-Mann (8). Since mucosa disintegrates rapidly when separated from the muscular layers, we used strips of the whole wall of the caecum cut to about 2 to 3 by 30 mm. in size and weighing 50 to 100 mgm. The tissue was blotted with filter paper, the wet weight was measured, and it was then used immediately for metabolic studies. The ratio of wet weight to dry weight was determined upon similarly treated strips from the same organ, dried at 110° C. for two hours.

Oxygen uptake and anaerobic glycolysis were studied by standard manometric methods (Dixon (3)), with Suhmerson differential manometers attached to flasks of about 15 ml. capacity. All runs were made in replicate at 41° C. for two hours, with shaking at a rate of 120 oscillations per minute and 4 cm. amplitude. In experiments with sulphamerazine the sodium salt was added in amounts sufficient to give a concentration of 0.001 M in the medium.

Tissue Thickness and Dry Weight

The mean thickness of whole caecal wall was 1.00 ± 0.09 mm. (eight determinations).* The dry weight was found to be 17.6 ± 0.2% of the wet weight (26 determinations). Invasion of the mucosa by parasites resulted in congestion and swelling of the caecal wall (up to 2.0 mm.), but did not change the percentage of dry weight.

In a few experiments the mucosa stripped from the muscular wall was used. The thickness of the mucosa alone was 0.3 to 0.5 mm., and the dry weight averaged 16.2 ± 0.4% of the wet weight.

Oxygen Uptake

The limiting thickness of tissue for maximum oxygen uptake has been estimated as 0.5 mm. (3), and the thickness of the caecal wall exceeded this limit. Preliminary experiments indicated, however, that respiration in air was only 25% less than in pure oxygen; and as the greatest metabolic activity resided in the mucosa it was believed that the results were not greatly affected by the thickness of the tissue strips.

All subsequent respiration determinations were run in pure oxygen. The rate of oxygen uptake did not remain constant over the two hour period, but fell off slightly from the linear, as indicated in the result of a typical experiment:—

40 min............... −110 µl. oxygen
80 min............... −210 µl. oxygen
120 min............... −296 µl. oxygen

* *All averages are reported as mean values ± the standard error of the mean.*

The Q_{O_2} values were calculated from oxygen uptake in the first 60 min.; and a summary of 14 determinations (five birds) gave a mean Q_{O_2} of -7.35 ± 0.45. Results with parasitized birds are summarized in Table I, from which it is evident that the presence of parasites did not increase the rate of oxygen consumption. (In one experiment upon normal mucosa stripped from the muscular wall, the values obtained were: -9.4, -9.8, -10.3.)

TABLE I

RESPIRATION OF NORMAL AND PARASITIZED CHICK CAECUM

Strips of whole caecum in 2.0 ml. of Krebs-Eggleston (7) phosphate saline, pH 7.4, containing 0.2% glucose. Gas phase oxygen, potassium hydroxide-filter paper in center well; 60 min. at 41° C

Days after infection	Stage of parasite development	Condition of mucosa	Q_{O_2} (replicates)
0	—	Normal	-7.35 ± 0.45
1	First stage schizonts	—	-7.0, -7.2
3	Developing schizonts	—	-6.8, -6.8
4	First generation merozoites	Slight haemorrhage	-5.4, -7.8
5	Second generation merozoites	Acute haemorrhage, congestion	-4.6, -4.7

A concentration of 0.001 M sulphamerazine in the medium did not affect the respiration of either normal or parasitized tissue.

Anaerobic Glycolysis

Carbon dioxide production plotted against time gave a rate that was virtually constant for the two hour period or that fell off very slightly. The carbon dioxide production in the first 60 min. was used for calculating the values of $Q_G^{N_2}$.

The values for anaerobic glycolysis in whole caecum, normal and parasitized, are summarized in Table II. Sulphamerazine at 0.001 M concentration had no effect. A slight increase in glycolysis at the three day stage of parasite development is suggested. A few experiments upon mucosa alone also appear to indicate such an increase, although the difference is not statistically significant.*

Discussion

Caecal tissue, parasitized with *Eimeria tenella* at various stages of development, showed no increase in rate of respiration as compared with normal tissue; in fact, there was a slight decrease in respiratory activity in the acute stages of the disease, when the mucosa was congested and haemorrhagic.

* *The difference in the mean values for normal and parasitized mucosa is 5.0 $\pm$ 2.1. Hence the t value is 2.4, giving a probability of 0.05 to 0.10.*

TABLE II

ANAEROBIC GLYCOLYSIS OF NORMAL AND PARASITIZED CHICK CAECUM

Strips of caecal tissue in 2 0 ml. of calcium-free Krebs-Henseleit Ringer-bicarbonate (3) containing 0.2% glucose. Gas phase 5% CO_2 + 95% N_2; 60 min. at 41° C.

Days after infection	Stage of parasite development	Condition of mucosa	$Q_G^{N_2}$ (replicates)
Whole wall 0	—	Normal	7.1, 7.6 7.2, 7.5
3	Developing schizonts	—	9 2, 9.8 8 0, 8.1, 8.2
4	First generation merozoites	Marked haemorrhage	7.0, 7.6
7	Gametocyte	Very acute haemorrhage	6.3, 6.4
Mucosa alone 0	—	Normal	8 9, 10.0 9 5, 10.8, 12.9
3	Developing schizonts	No haemorrhage	11.5, 16.7, 17.9

Determination of anaerobic glycolysis revealed a slight but statistically insignificant increase at the third day after infection, especially when mucosa alone was used. A larger number of experiments would probably reveal a significant increase in the parasitized tissue.

When these results are compared with the findings obtained with blood parasitized with *Plasmodium*, it is evident that normal erythrocytes have a very low metabolic rate, which is notably increased by the presence of the parasites. In the case of intestinal tissue, on the other hand, the tissue itself possesses an active metabolism, and any additional activity due to the parasites is scarcely measurable.

Sulphamerazine is a strongly coccidiostatic drug, and might be supposed to inhibit the metabolism of the coccidia; but since the additional activity due to the parasites was not measurable, no effect of the drug could be observed. Furthermore, the low concentration of sulphamerazine in the medium may not penetrate to the parasites. If it were possible to measure a significant increase in glycolysis, a better method of investigating the effect of the drug would be to administer it to the chicks by mouth, in therapeutic doses, and then to measure the metabolic activity of caecal tissue after a suitable interval. The limited time available for this project precluded the carrying out of such experiments.

The values obtained for metabolism of chick caecal tissue were of the same order of magnitude as those observed with mammalian gastrointestinal tissue, as put forth in Table III. It may be estimated from our data that about two-thirds of the metabolic activity of the caecal tissue resides in the mucosa, although it occupies less than one-half of the total thickness.

TABLE III

COMPARISON OF METABOLIC ACTIVITY OF CHICK CAECUM AND MAMMALIAN GASTROINTESTINAL TISSUE

Tissue	Q_{0_2}	$Q_G^{N_2}$	Author
Chick caecum			
Whole wall	− 7.4	7.4	Collier and Swales
Mucosa	− 9.8	10.4	
Rat jejunum*			
Whole wall	− 7.8	8.1	Dickens and Weil-
Mucosa	−11.3	12.6	Malherbe (2)
Rat stomach†			
Whole wall	−8 to −15	11	Lutwak-Mann
Mucosa	−10	6	(8)

* Based upon the first 20 min. period.
† Estimated from the graphs (60 min. values).

References

1. CHRISTOPHERS, S. R. and FULTON, J. D.   Observations on the respiratory metabolism of malaria parasites and trypanosomes.   Ann. Trop. Med. 32 : 43-75.   1938.

2. DICKENS, F. and WEIL-MALHERBE, H.   Metabolism of normal and tumor tissue.   19. The metabolism of intestinal mucous membrane.   Biochem. J. 35 : 7-15.   1941.

3. DIXON, M.   Manometric methods as applied to the measurement of cell respiration and other processes.   2nd ed.   Cambridge Univ. Press, London.   1943.

4. EVANS, E. A., JR.   Enzyme systems operating within the malarial parasite.   Federation Proc. 5 : 390-396.   1946.

5. FARR, M. M. and WEHR, E. E.   Developmental stages in the life cycle of *Eimeria tenella* affected by sulfamethazine treatment.   Proc. Helminth. Soc. Wash., D.C. 14 : 12-20. 1947.

6. HELLERMAN, L., BOVARNICK, M. R., and PORTER, C. C.   Metabolism of the malaria parasite.   Action of antimalarial agents upon separated *Plasmodium lophurae* and upon certain isolated enzyme systems.   Federation Proc. 5 : 400-405.   1946.

7. KREBS, H. A. and EGGLESTON, L. V.   The oxidation of pyruvate in pigeon breast muscle.   Biochem. J. 34 : 442-459.   1940.

8. LUTWAK-MANN, C.   Metabolic processes in the gastric mucosa.   Biochem. J. 41 : 19-28. 1947.

9. McKEE, R. W., ORMSBEE, R. A., ANFINSEN, C. B., GEIMAN, Q. M., and BALL, E. G.   Studies on malarial parasites.   VI. The chemistry and metabolism of normal and parasitized (*P. knowlesi*) monkey blood.   J. Exptl. Med. 84 : 569-582.   1946.

10. RIPSOM, C. A. and HERRICK, C. A.   Effects of various sulfa compounds on the protozoan parasite *Eimeria tenella*.   J. Parasitol. 31 : 98-108.   1945.

11. SILVERMAN, M., CEITHAML, J., TALIAFERRO, L. G., and EVANS, E. A., JR.   The *in vitro* metabolism of *Plasmodium gallinaceum*.   J. Infectious Diseases, 75 : 212-230.   1944.

12. SWALES, W. E.   On the chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens.   Can. J. Research, D, 22 : 131-140.   1944.

ACCESSORY TAILS IN FROG TADPOLES, THEIR EXPERIMENTAL PRODUCTION AND SIGNIFICANCE

II. SOME EXPERIMENTAL METHODS OF PRODUCING ACCESSORY TAILS IN FROG TADPOLES[1]

By David J. McCallion[2]

Abstract

Under certain conditions of injury to the tail of the tadpole of *Rana clamitans* accessory tails arise by regeneration. Methods of obtaining these conditions are outlined and the resultant accessory tails are described. Previous literature dealing with the mechanism that initiates the regenerative process in frog tadpoles is discussed and interpreted in the light of evidence gained in studies of accessory tail formation.

Introduction

Of the many problems involved in regeneration, one of the more significant is that of internal influence. There is a vast and often confusing or conflicting literature dealing with the causative influences of various structures on the regeneration of a part in almost all the major groups of animals. In some cases the problem has been adequately dealt with. In others the results are not so satisfactory.

The present paper is largely concerned with the relative influence of the nerve cord and the notochord on the regeneration of the tail of the frog tadpole. Does one, or both, or indeed either, of these structures initiate the regenerative process or induce the formation of a tail? If these structures influence tail regeneration, is such influence a contact principle, effective only when these structures are present at the cut surface, or a diffusion principle that becomes less effective with distance from the cut surface?

Experimental studies of growth and regeneration in very early stages of development in frog larvae (by Barfurth, Goldstein, Braus, Harrison, and others) seem to indicate that the central nervous system does not exercise any morphogenetic influence on the development or regeneration of embryos. Other investigators (Morgan and Davis (11), Wintrebert (15), and Goldfarb (6)) have attempted to show that the nerve cord has no influence on the regeneration of the tail in older tadpoles. In some respects their results were not entirely satisfactory.

The experiments of Morgan and Davis consisted in amputation of the distal one-third of the tail, together with the extirpation of a short length of the nerve cord, or notochord, or both. The muscle masses on either side were necessarily removed at the same time. Wintrebert's experiments were somewhat similar. A portion of the tail was amputated and the stump was

cut in several ways in order to produce two branches, or three branches in which nerve cord, notochord, and aorta were separated or variously combined. The results of both these sets of experiments were quite varied and not always in agreement with those of later investigators. However, they were interpreted as indicating that regeneration of the tadpole tail is independent of the nerve cord but dependent upon the notochord.

It has been stated that the results of these experiments are not in agreement with those of later workers. Goldfarb (6) and others have shown that the tail of the frog tadpole is innervated from ganglia lying in the anterior half of the tail. There are no ganglia in the distal half of the tail. It is obvious, then, that mutilations of the tail, as described above, interfere very little with the innervation of the stump. More important, however, is the difficulty of separating the nerve cord from the notochord. They are very closely applied to each other and the muscle masses surrounding them are opaque. As a result of this difficulty, fragments of nerve cord (and notochord) are usually present in the isolated parts. These fragments are themselves capable of regeneration. Therefore, although these experiments have tested, in some measure, the value of the intact nerve cord, they do not test the value of regenerating fragments nor of peripheral fibers.

When Tornier (14) repeated the experiments of Morgan and Davis he got no regeneration at all. He attributed this to physiological inhibition of regeneration exerted by the overhanging muscle masses. Later, Avel (1) showed that this was merely mechanical obstruction of regeneration.

The writer also attempted to repeat these experiments. After unsuccessful attempts to separate the notochord and nerve cord without injury to either, the distal one-third of the tail was amputated and sufficient tissue to include both nerve cord and notochord was cut out for a distance of several mm. (Fig. 1a). In only one of 10 cases studied did the overhanging muscles

Fig. 1. *A line drawing to show the nature of the injury to the tail (a) after the method of Morgan and Davis (see text), (b) after the method of Wintrebert (see text).*

meet and fuse, and thus block regeneration of the notochord. In the other cases the notochord grew through the cut region and at least five millimeters beyond the amputation level. These observations are in agreement with those of Avel and contrary to those of Tornier. Regeneration of the nerve cord was irregular but it was carried along with the notochord. The one important observation was that the upper and lower masses of muscle and connective tissue showed a small amount of regeneration (about 2 mm.) although some distance from the cut ends of the nerve cord and notochord.

Similarly, when the tail stump was split into three parts (after Wintrebert, Fig. 1b) it was never certain that the nerve cord and notochord were cleanly

separated.   In every case a new tail regenerated from the middle piece.   In most cases the outer parts became more or less fused with the central part. In those few cases in which either the upper or lower part remained free, and therefore, distant from the notochord at least, these parts showed some regeneration but never produced tails.

Having shown that the tip of the tail was innervated from ganglia at more proximal levels, Goldfarb attempted to denervate the tail in several ways, especially by transection of the nerve cord at the base of the tail.   This usually resulted in such extensive injury or mutilation that the animals died. In those that survived he was never sure of a denervated stump, but from what results he got, he thought that in all probability the central nervous system has no influence on tail regeneration in frog tadpoles.   On the other hand, he had already satisfied himself that, in adult urodeles, the determining factor in the formation of a tail is the presence of the nerve cord at the cut surface.

More exact studies on the influence of the notochord in development and regeneration have been possible.   It has been demonstrated by Politzer (12) and Risley (13) that notochordal tissue has some influence in inducing embryonic tail formation in urodeles.   Kollmann (8) and Francescon (4) showed that, if in frog tadpoles the notochord was destroyed or partially destroyed, and therefore, prevented from regenerating, the tail did not regenerate.   Morgan and Davis (11) also claimed that the presence of the regenerating notochord at the amputation surface was essential to tail regeneration.   Wintrebert (15) was of the opinion that it is the reconstitution of the notochord as a supporting axis, as much as any influence it might have, that regulates tail regeneration.

Accessory tails resulting from natural and experimental injury have been frequently recorded in lizards, urodeles, and frog tadpoles.   In an earlier paper by the writer (McCallion (9)) five cases of accessory tails in frog tadpoles arising from natural injury were described.   Varying in degree of perfection, they have been produced in frog tadpoles by a number of investigators.

Harrison (7), using Born's method of grafting, obtained forked tails in *Rana palustris* tadpoles.   He severed the tails from two young larvae and united the larvae in such a way that their tail stumps met with their axes at an angle of about 135°.   Later the notochord of one member of the pair had regenerated ventrally into the ventral fin fold of the other.   This notochordal growth was accompanied by muscle and blood vessels.   At this time the animals were cut apart so that the principal larva had a forked tail.   In this manner, Harrison obtained 10 instances of bifid tails, a few of which also had a forked nerve cord.   These structures are not, in the sense of this discussion, accessory tails, but the tail of one animal grafted upon the tail of another.   Even though the grafts took well, the two notochords never became united.

Barfurth (2) tried to obtain bifid tails in *Rana fusca* tadpoles by splitting the tail into upper and lower parts, but with slight success. He was successful in producing such structures by a double injury to the tail. A hot needle was thrust into the side of the tail followed by amputation just posterior to the first injury. Subsequent regeneration produced a tail from each of the two sites of injury. By a somewhat similar procedure Avel (1) obtained similar results with *Rana temporaria*. The side of the tail was pierced in such a manner as to remove a short piece of notochord, but leaving the tail end attached. A small tail regenerated from the site of injury, assumed the normal axis, and pushed the old tail aside.

Material and Methods

Young *Rana clamitans* tadpoles, collected from a pond near McMaster University, were used in these experiments. They were kept in the laboratory in large numbers with considerable success. Animals used in experiments were first anaesthetized in Chloretone solution (1 : 1000 water). The cuts were made as quickly as possible with a fragment of razor blade fixed to a glass handle. After operation the animals were placed in Ringer's solution for 24 hr., and transferred to tap water for six weeks or longer. After that time the tails of these animals were photographed and prepared for histological examination.

Experiments and Results

METHOD I

A simple method of obtaining accessory tails in frog tadpoles is by incomplete amputation. If the tail is incompletely amputated by a single incision, it is difficult to prevent the edges of the wound from healing together. To overcome this difficulty V-segments or long segments were cut out from the dorsal edge of the tail in some cases (Figs. 2a, 2b), and from the ventral edge in others (Figs. 2c, 2d). Sufficient tissue was removed to include the notochord and this was, in effect, the same as an incomplete amputation. Forty-two cases were studied.
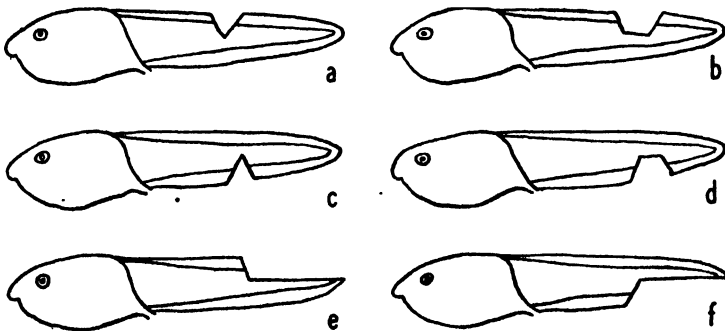
Fig. 2. *Line drawings to show the amounts of tissue removed from the edge of the tail in Method I; a and c show V-segments, b and d long segments, e and f show the location and amount of tissue removed in Method II.*

In a small number of cases the old tail end was torn away by the activity of the tadpoles. In these cases normal terminal regeneration followed. In a few instances the injury was not made sufficiently deep to include the notochord, and that portion of the tail that was removed was regenerated and a nearly normal structure resulted.

If the injury was made from the dorsal edge of the tail, and in such a way that the notochord was severed, regenerative growth proceeded from both surfaces of the cut. When the injury was sustained near the tip of the tail the posteriorly directed regenerate became an accessory tail (Figs. 3, 4, 5). It was more or less complete, lacking only a ventral fin fold. This structure possessed nerve cord and notochord accompanied by some muscle fibers. The caudal vessels, if present, were very irregular.

The nature of the anteriorly directed regenerate depended upon the location of the injury. Morgan (10) observed that if a cut was made into the dorsal edge of the tail near its base, regeneration from the anterior surface of the wound was slight but a heteromorphic tail arose from the posterior surface. It was noted in these experiments that if the injury was made in the middle regions of the tail growth from the opposing surfaces of the wound was nearly equal in rate and amount and the regenerates overlapped (Fig. 6). At the tip of the tail regeneration from the anterior surface was greater and an accessory tail resulted (Fig. 4).

Similar injuries to the ventral edge of the tail gave substantially the same results. There was regeneration from both surfaces of the wound when the notochord was severed. When the notochord was not injured a nearly normal tail resulted.

In these cases, however, the nature of the accessory tail (Figs. 7, 8) depended upon the extent of the injury. If the cut was sufficiently deep to injure the nerve cord, both nerve cord and notochord appeared in the regenerate. If the nerve cord was not injured it was not observed in the accessory tail. Muscle fibers were always present, but less than the normal amount. The regenerating caudal vessels produced many small branches and were, therefore, small and irregular in the regenerate.

Method II

Thirty cases were studied in the following manner. In half the cases the dorsal half of the distal one-third of the tail was cut out in such a way as to include approximately half of the notochord (Fig. 2e). In the other half of the cases similar amounts of tissue were removed from the ventral side of the tail (Fig. 2f). Subsequent regeneration was dependent upon how the cuts removing the tissue were made.

If the transverse cut was made nearly perpendicular to the notochord, or at an obtuse angle with it, an accessory tail regenerated from that surface of the wound (Figs. 9a, 9b; 10a, 10b). If, however, this cut was made so that the exposed surface made an acute angle with the long surface of the injury,

PLATE 1
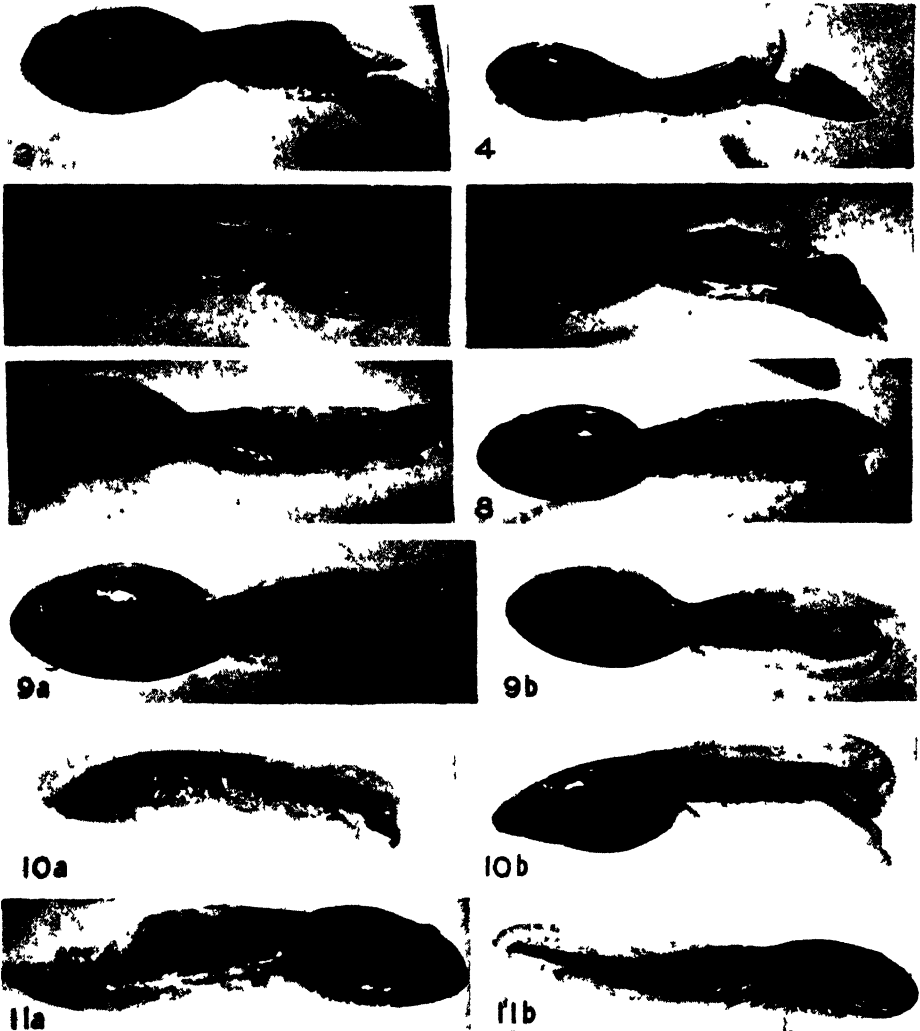
FIGS 3 4 AND 5   Photographs (about 1 0×) to show the dorsal accessory tails produced following the injuries shown in Figs 2a and 2b   Note the slight regeneration from the posterior surface of the wound in Figs 3 and 4

FIG 6   Photograph (about 1 0×) to show the overlapping regenerates when the injury was nearly in the middle regions of the tail

FIGS 7 AND 8   Photographs (about 1 0×) showing the ventral accessory tails regenerated from the ventral injuries shown in Figs 2c and d

FIG 9   Photographs (about 1 0×) to show the dorsal accessory tails following the removal of tissue as shown in Fig 2c

FIG 10   Same to show ventral accessory tails   Fig 10a is a poor photograph but is used here to show a distinct but peculiar accessory tail

FIG 11   Photographs (about 1 0×) showing the nearly normal tails resulting when the notochord is not injured by the operation   Note over regeneration of the ventral fin in b   In a the notochord had been injured slightly and had regenerated dorsally   The notochord is the dark line in the dorsal fin near the tip of the tail

NOTE   In the photographs the regenerates can be easily distinguished by the absence of pigmentation in the new tissues

PLATE II
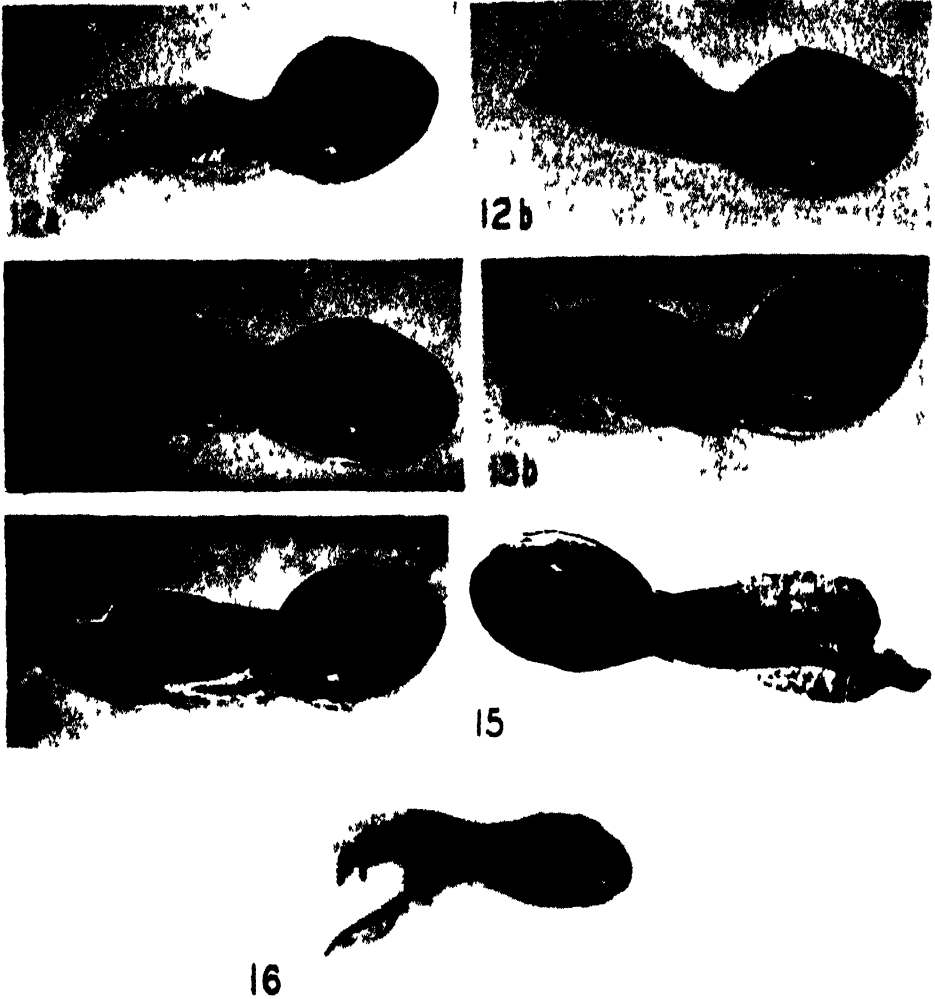
FIG. 12   Photographs (about 1 0X) to show the bifurcated tails following the operation shown in Fig 18a   The central branch of the tail was the first regenerate

FIG. 13   Same showing the nature of regeneration following the operation seen in Fig 18b   The dorsal branch was the first regenerate

FIG. 14   Photograph (about 1 0X) to show that the upper branch of the split tail degenerated when deprived of notochord   Note that the lower branch is nearly complete

FIG. 15   Same showing partial degeneration of the upper part, and regeneration of the lower part of the split tail

FIG. 16   Same showing that both branches persist and regenerate when each contains a part of the notochord.

the part removed was replaced by regeneration and a nearly normal tail resulted. Similarly, nearly normal tails were obtained if the notochord was uninjured (Figs. 11*a*, 11*b*).

In those cases that produced accessory tails from the transverse surface of the wound the old tail end was completed by regeneration from the long surface.

When the injury was made dorsally the nerve cord was injured in every case but the caudal vessels were not. The resultant accessory tails possessed a nerve cord, a notochord, and some muscle, but no definite caudal vessels. The ventral fin was usually lacking. The old tail end contained a notochord completed by regeneration and the original caudal vessels. In some cases fragments of nerve cord were accidentally left behind; these regenerated to produce a more or less complete nerve cord.

In one case the notochord was not injured but a regenerating tube of skin that had become infolded in some way served as a supporting axis for the accessory tail (Fig. 17). Outwardly, this regenerate did not appear different from the others.

When the ventral part of the tail was removed the transverse surface of the injury again produced an accessory tail and the old tail end was repaired by regeneration from the long surface. In most cases the nerve cord was not injured, but in a few cases the accessory tail contained a nerve cord when that structure had been accidentally severed. Otherwise, only the notochord was present. In every case definite but irregular caudal vessels appeared in the regenerate. Usually the dorsal fin was lacking. The old tail end contained the nerve cord and a notochord completed by regeneration. Definite caudal vessels were absent. As before, some muscle fibers were present in both the accessory tail and the old tail end.

Method III

Efforts to obtain accessory tails by a double amputation of the tail were made on 60 tadpoles. This method was a modification of that used by Dawson (3) to obtain a ventral accessory tail in *Triturus viridescens*.

In half of these cases the tip of the tail was amputated obliquely (Fig. 18*a*) so that the injured surface was directed ventrad. Resulting terminal regeneration produced a ventrally directed tail tip. When the regenerate was well established but before it had become regulated to its normal axis, a portion of the dorsal edge of the tail was removed at the point of deflection of the new tail tip (Fig. 18*a*). The ventrally directed regenerate continued its growth and a dorsal accessory tail was produced from the site of the second injury (Figs. 12*a*, 12*b*). This method was successful in relatively few cases because of the difficulty of making the second injury at just the right time and location. To ensure success the first regenerate had to be considerably deflected and the second injury made at just the angle of deflection and deep enough to injure the notochord. In most cases failure to obtain accessory tails was caused by the rapid regulation of the first regenerate to its normal axis.

In all cases in which accessory tails were obtained the ventral branch was complete since it was a normal terminal regenerate. The dorsal branch of the tail contained a nerve cord and a notochord accompanied by muscle fibers. Blood vessels were present but very irregular. Both branches of the tail were usually contained within a common fin fold.
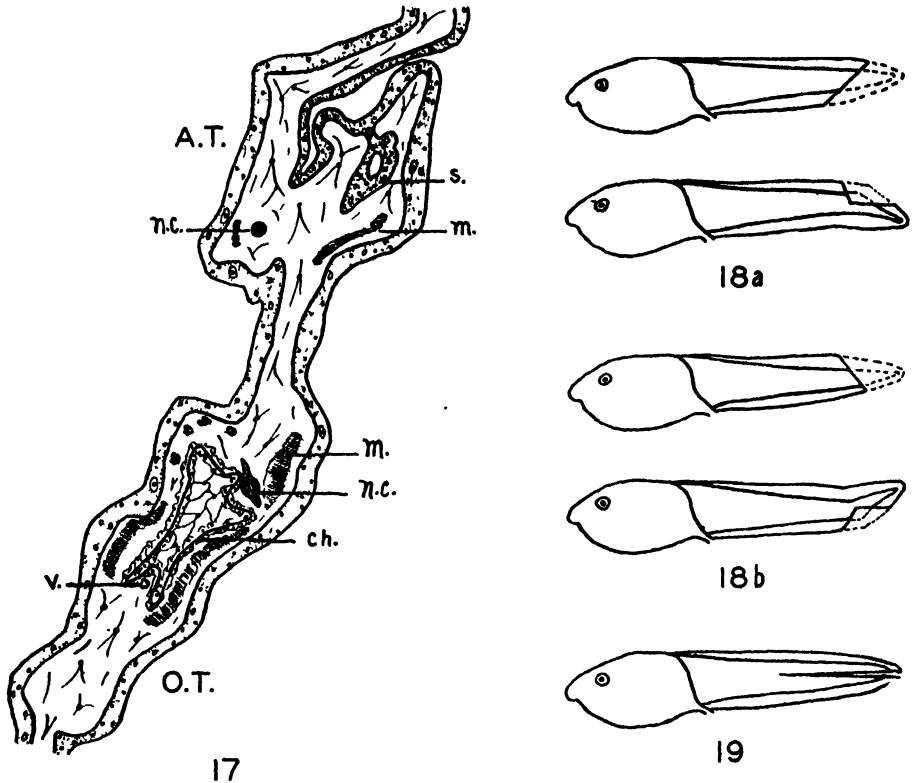
FIG. 17.   *Camera lucida drawing (ca. 15×) of a transverse section of the tail of a tadpole such as that shown in Fig. 9a, b showing that the accessory tail in one case was supported by a fold of skin.*
    A.T. = *accessory tail;* O.T. = *old tail;* m. = *muscle;* s. = *infolded tube of skin;* n.c. = *nerve cord;* ch. = *notochord;* v. = *caudal vessel.*
    FIG. 18.   *Line drawings to show the two successive operations described in Method III. Dotted lines indicate amount of tissue removed.*
    FIG. 19.   *Line drawing to show how the tail was split longitudinally in Method IV.*

Similar results were obtained and the same difficulties encountered if the tip of the tail was amputated so that the amputation surface was directed dorsad (Fig. 18b) and a portion of the ventral edge of the tail was cut out at the angle of deflection (Fig. 18b).

When bifid tails were obtained in these cases (Figs. 13a, 13b) the upper branch was the first regenerate and therefore complete. The lower branch regenerated from the site of the second injury and its nature depended upon the extent of that injury.   If the second cut was sufficiently deep to injure

the nerve cord, it regenerated and appeared in the lower branch. Otherwise, the nerve cord could not regenerate and was, therefore, absent from the lower branch. In all cases the ventral branch of the tail contained a notochord, muscle, and irregular vessels. Both branches of these tails were also contained within a common fin fold.

METHOD IV

One of Barfurth's (2) experiments was repeated on 15 tadpoles with a small measure of success. The tail was split longitudinally, parallel to the lateral line (Fig. 19). An attempt was made to split the notochord in half, whereas Barfurth attempted to separate the nerve cord from the notochord. Such a condition is extremely difficult if not impossible to obtain because of the close relationship between these structures and the opacity of the muscle mass.

A variety of results were obtained from this experiment. In many cases the halves of the tail healed together and the results were unsatisfactory. In two cases the dorsal portion of the split tail was deprived of notochord by the operation. In both cases, the upper part shrivelled and degenerated (Fig. 14), even though some nerve cord was present. The ventral portion regenerated a nearly normal tail including a dorsal fin.

In some instances the notochord was successfully split nearly in half. Under these conditions both halves of the tail not only persisted but regenerated (Figs. 15, 16). The upper portion contained a nerve cord, and a notochord completed by regeneration, and muscle fibers. The lower half contained a completed notochord, muscle, and caudal vessels. When the cut was irregular and fragments of nerve cord were left behind in the lower portion of the tail, a new nerve cord regenerated from the fragments.

In at least one case the notochord was isolated from the upper portion of the tail but a scar of skin tissue formed a sufficient support to maintain that part.

SURVEY OF RESULTS

Accessory tails or bifid tails are relatively easily produced in *Rana clamitans* tadpoles, and possibly in those of other species. It is interesting to note the great capacity for regeneration in this structure, particularly in view of the absence of regenerative capacity in the adult. It was frequently observed in these experiments that a great deal more tissue was produced by regeneration than was lost by injury, although this is not usually true in cases of normal terminal regeneration. The notochord is particularly capable of over-regeneration. This phenomenon probably accounts for the production of accessory tails. These observations support the opinion held by Barfurth that an accessory tail is an independent product of regeneration and not an incomplete reduplication of the tail.

Accessory tails produced by experimental methods were never histologically perfect and not always complete. The caudal vessels were always abnormal and atypically branched. This condition has also been reported by Fukai (5)

for other species of frog and toad tadpoles. The notochord was rarely cylindrical but showed irregular contours even when great care was taken with the preparation of the tails for study. Muscle was never as abundant in the accessory tail as it was in the old tail. The nerve cord frequently showed several branches, particularly when it was derived from fragments.

However, accessory tails always contained a notochord and muscle fibers, were nourished and innervated in some way, and were, therefore, functionally adapted. As such they are true tails, independent products of regeneration, and not simply reduplications of the tail.

Accessory tails arise only when the notochord has been previously injured so that it can regenerate in two directions or in two places simultaneously, except in those cases in which the old tail end was incompletely severed. In a very small number of cases a fold of skin had served as a supporting axis for an accessory tail in the absence of the notochord. Accessory tails can be produced in the absence of injury to the nerve cord, that is, in the absence of a regenerating nerve cord.

Discussion

In most groups of animals in which experiments have been carried out it has been acceptably demonstrated that regeneration of a part is dependent upon the presence of at least some nerve fibers at the cut surface. In the work cited in the Introduction none of the authors have obtained a completely nerveless tail stump. There is no direct evidence that regeneration of the tail of the frog tadpole is independent of the nervous system. Repetitions or modifications of the same experiments have been equally unfruitful. The writer's experiments show that under certain conditions of injury accessory tails arise when the nerve cord itself has not been injured. Such accessory formations, in most cases, resembled a normal tail end but were lacking a central nerve cord. It has already been stated that the distal half of the tail is innervated from ganglia situated in the proximal half. It is obvious, then, that there were always nerve fibers present at the cut surface even though the nerve cord had not been injured at that region. It may be justly inferred from this evidence that regeneration of the distal portion of the tail of the frog tadpole is independent of the presence of a cut central nerve cord at the amputation surface; that this structure neither initiates the regenerative process nor induces the formation of a tail. However, there is no evidence to suggest that this phenomenon is entirely independent of peripheral nerves. There is also the possibility that some influence is diffused from the intact nerve cord that becomes less active with distance from it.

It has been more clearly demonstrated that notochordal tissue induces tail formation in embryos of urodeles and anurans. Experiments on older anuran larvae seem to indicate that the presence of the notochord at the cut surface is essential to direct regeneration of the tail. The work of Wintrebert is probably the most significant. The results of his experiments seem to indicate that it is not merely the presence of the notochord but its function

as a supporting axis that is involved in regulating tail regeneration.   Accessory tails arise only when the notochord has been severed or partially severed, with the possible exception of one or two cases (Fig. 17) in which a fold or tube of skin functioned in lieu of the notochord.   When the notochord was not injured a nearly normal tail was regenerated.   This fact supports Wintrebert's point of view that the notochord is essential to tail regeneration in frog larvae as a supporting axis around which new tissues are produced to form a tail.

Conclusions

It is generally accepted that the tissues of the tadpole tail in regeneration are derived from previously existing tissues by a process of dedifferentiation, properly called modulation.   That is, new muscle is derived from preëxisting muscle, nerve cord from nerve cord, and notochord from notochord, and not from an indifferent blastema.   Studies of accessory tail formation further support this point of view.

Any structure that is isolated from a part of the tail by mutilation does not appear in the regenerate.   Accessory tails can be produced that lack a central nerve cord, notochord, or caudal vessels.   This is taken to mean that, if any of these structures are isolated from the injured region, the regeneration blastema formed at the injured surface does not contain elements for their formation.   In other words, the blastema is not composed of indifferent elements.   For this reason, injury that includes only muscle and connective tissue, with some nerves and small blood vessels, does not produce an accessory tail, since there is no source of a supporting notochord.

Since there is some regeneration of tail tissues in the absence of injury to the notochord, it is obvious that regeneration is not dependent upon the presence of the notochord at the cut surface.   The notochord does not initiate the regenerative process but its function as a supporting axis secondarily directs the formation of a tail and therefore is essential to the regeneration of a tail.   From the evidence available it may be inferred that regeneration in the distal part of the tail is independent of the central nerve cord.   There is no evidence to suggest that the peripheral nervous system as represented by nerve fibers at the cut surface has no influence on the process of regeneration, although it has been assumed by some that the influence of the nervous system is probably negligible.   However, in view of the evidence obtained in studies of regeneration by many careful and methodical workers, in both invertebrates and vertebrates it cannot be said with certainty that regeneration of the tail of the frog tadpole is entirely free of some influence of the nervous system. Further experimentation is necessary to determine the extent of nervous influence and to explore the possibility of a histochemical influence (perhaps active through nerve fibers) on tail regeneration in frog tadpoles.

Acknowledgment

References

1. AVEL, M.   L'inhibition de la régénération de la corde dorsale chez les têtards des amphibiens anoures.   Compt. rend. soc. biol. 109 (5) : 693-695.   1932.

2. BARFURTH, D.   Die experimentelle Herstellung der Cauda Bifida bei Amphibienlarven.   Arch. Entwicklungsmech. Organ. 9 : 1-26.   1899.

3. DAWSON, A. B.   A ventral accessory tail in *Triturus viridescens* and its duplication experimentally.   Anat. Rec. 52 (2) : 139-145.   1932.

4. FRANCESCON, A.   Rigenerazioni della corda dorsale.   Monitore Zool. Ital. 40 (11/12) : 431-433.   1930.

5. FUKAI, TATSUO.   Über den Verlauf des Blutgefässes am regenerierten Schwanz von Amphibienlarven.   Folia Anat. Japon, 12 (4) : 223-228.   1934.

6. GOLDFARB, A. J.   The influence of the nervous system in regeneration.   J. Exptl. Zool. 7 (4) : 643-722.   1909.

7. HARRISON, R. G.   The growth and regeneration of the tail of the frog larva.   Arch. Entwicklungsmech. Organ. 7 : 430-481.   1898.

8. KOLLMANN, M.   Régénération caudale chez les Batraciens.   Régulation et régénération.   Compt. rend. soc. biol. 85 : 1007-1010.   1921.

9. McCALLION, D. J.   Accessory tails in frog tadpoles, their experimental production and significance.   I. Five cases of accessory tails in tadpoles of *Rana clamitans* as a result of natural injury.   Can. J. Research, D, 26 : 62-65.   1948.

10. MORGAN, T. H.   Regeneration.   The Macmillan Company, New York and London.   1901.

11. MORGAN, T. H. and DAVIS, S. E.   The internal factors in the regeneration of the tail of the tadpole.   Arch. Entwicklungsmech. Organ. 15 : 314-318.   1903.

12. POLITZER, G.   Teratologische Untersuchungen zur Entwickelungs nach der Schwanzknopfe.   Arch. Entwicklungsmech. Organ. 115 (4/5) : 636-650.   1929.

13. RISLEY, P. L.   An experimental study of anus formation in *Urodela*.   J. Exptl. Zool. 80 (1) : 113-128.   1939.

14. TORNIER, G.   Experimentelles und Kritisches über tierische Regeneration.   Sitzber. Ges. naturforsch. Freunde Berlin.   50-66.   1906.

15. WINTREBERT, P.   Sur la valeur comparée des tissus de la queue au point de vue de la régénération chez les larves d'Anoures et sur l'absence possible de cette régénération.   Compt. rend. 139 : 432-434.   1904.

A COMPARATIVE STUDY OF THE PROVENTRICULUS OF ORTHOPTEROID INSECTS WITH REFERENCE TO ITS USE IN TAXONOMY[1]

By W. W. Judd[2]

Abstract

The structure of the proventriculus in 115 species of orthopteroid insects (eight orders) is investigated.

The Blattodea, Mantodea, and Isoptera have a conical proventriculus, with 6 or 12 longitudinal teeth. There are eight tubular gastric caeca. The Ensifera (Grylloidea and Tettigonioidea) have a globular proventriculus with a tubular neck. In the globular part are six longitudinal folds each bearing a series of appendages and separated from one another by partitions. There are two bulbous gastric caeca. The four families of the Caelifera have a tubular proventriculus. The Acrididae have six longitudinal plates in the proventriculus and six gastric caeca with anterior and posterior projections. The other three families have no plates in the proventriculus. The Tridactylidae have two gastric caeca, the Tetrigidae have six short, conical caeca, and the Cylindra-chaetidae have six long, tubular caeca.

The Phasmida have a tubular proventriculus with longitudinal, spine-bearing folds and a long flaplike oesophageal valve. In the Grylloblattaria the organ is globular with 12 longitudinal folds in the intima, and two ranks of 12 pyramidal teeth at its posterior end. The Dermaptera have a tubular proventriculus, flared slightly where it joins the crop. Internally there are six longitudinal folds bearing small scalelike projections, and a cushion of bristles at the anterior end of each fold. In the Plecoptera the proventriculus is tubular, with 14 longitudinal, spine-bearing plates on its inner surface. There are seven tubular gastric caeca.

A 'phylogenetic tree' demonstrates the relationships of the groups studied, and a systematic key is prepared.

Introduction

The proventriculus, as the term implies, is the region of the fore-gut that lies immediately in front of the ventriculus or mid-gut of insects. In common with the rest of the fore-gut or stomodaeum it is ectodermal in origin, being lined with a sclerotized intima, in contrast to the mid-gut, which is endodermal in origin and which has no sclerotized intima. As will be shown in subsequent descriptions, the proventriculus displays all degrees of development from a simple valve lined with soft cuticle to a powerful muscular organ armed with spines and teeth.

While the term proventriculus is properly applied only to the posterior region of the fore-gut, some authors use it with reference to a swollen region of the mid-gut of Diptera. Snodgrass (105) shows that this is not truly a proventriculus since it is not ectodermal in origin and since the oesophageal valve projects into its anterior end, instead of projecting from its posterior end into the mid-gut. The work of several authors on Diptera confirms this

view (8, 38, 59, 60, 65, 72, 120). In some Diptera, notably in *Drosophila melanogaster*, the 'proventriculus' is composed of both ectodermal and endodermal tissues (96, 118). Among the Homoptera (Coccidae (74), Cicadidae (62, 63)) the region described as proventriculus is also of endodermal origin. In the Heteroptera the whole fore-gut is reduced in size (20, 58) although the oesophageal valve is well defined in some cases. It is thus to be seen that a specialized proventriculus in the fore-gut of sucking insects is rare. The most important exception to this generalization is in the Siphonaptera of which several species have a globular proventriculus lined with spines (19, 48, 86, 118).

It is in mandibulate insects that the proventriculus shows the widest diversity in structure. This wide diversity has led to investigations along three main avenues:

1. Correlation of the structure of the proventriculus with the feeding habits of insects.

2. Function of the proventriculus.

3. Study of the proventriculus as a structure to be used in classification of insects and in the investigation of phylogenetic relationships.

It is the purpose of the present writer to compare the structure of the proventriculus in various groups of orthopteroid insects with a view to determining what light this study may throw upon the phylogenetic relationships existing among these groups, and to investigate what use it may be in taxonomy. Conclusions concerning the phylogeny of insects have been based mainly upon consideration of the external anatomy, but, in the words of Walker (112), "in any attempt to unravel the relationships of a group of organisms it is obvious that the entire structure of the body should be taken into account".

System of Classification

In the 10th edition of his *Systema Naturae* Linnaeus (80) erected, in the order Insecta Coleoptera, three genera: *Forficula, Blatta*, and *Gryllus*, which correspond to the Dermaptera of DeGeer (39) and the Orthoptera of Olivier (85). To the first two of these he assigned a number of species and the last he divided into six subgenera: *Mantis, Acrida, Bulla, Acheta, Tettigonia*, and *Locusta*. Fabricius (49) adopted the generic names *Forficula* and *Blatta* and used the name *Gryllus* to designate Linnaeus' *Gryllus Locusta*, and the other five of the subgenera of Linnaeus' *Gryllus* he used as genera, changing the names of three of them: *Mantis, Truxalis (Acrida* L.), *Acrydium (Bulla* L.), *Acheta, Locusta (Tettigonia* L.). Stoll (106) adopted a classification similar to that of Linnaeus but divided the genus *Mantis* into two, *Mantis* and *Phasma*.

Leach (79) restricted the use of the name Dermaptera to the order of earwigs (Forficula) and included the cockroaches in the order Dictyoptera, the mantids in the order Mantida, and the walking-sticks in the order Phasmida. He assigned the crickets to a family of Achetidae, the long-horned

grasshoppers to a family Gryllidae, and the locusts to a family Locustidae. Burmeister (23) adopted a similar classification and used the following terms: Blattina (cockroaches), Mantodea (mantids), Phasmodea (walking-sticks), Gryllodea (crickets), Locustina (long-horned grasshoppers), Acridiodea (locusts). Karsch (77) adopted a classification similar to that of Burmeister but used the terms Phasgonuridea and Acridodea in place of Burmeister's Locustina and Acridiodea. Handlirsch (55, 56) in his exhaustive study of fossil insects established two superorders: Orthopteroidea and Blattaeformia. The former comprised the orders Orthoptera (suborders Locustoidea and Acridioidea), Phasmoidea, and Dermaptera and the latter the orders Mantoidea, Blattoidea, and Isoptera.

The inclusion of the Phasmoidea and Dermaptera with the Orthoptera in a superorder Orthopteroidea implies a close relationship among these three groups. That this relationship exists is disputed by some authors, and in this treatment the superordinal terms will not be used. The names of the orders adopted are then: Blattodea, Mantodea, Isoptera, Phasmida, Orthoptera (Saltatoria), Grylloblattaria, Dermaptera, and Plecoptera.

Ander's classification of the Saltatoria (1), based upon his phylogenetic studies has been adopted, and Roberts' (95) classification of the Acrididae based upon his study of phallic structures has been adopted.

The classification, to subfamily, of the forms investigated in this study is then as follows:

Order Blattodea
    Family Blattidae
        Subfamily Pseudomopinae
              "   Blattinae
              "   Panchlorinae
              "   Blaberinae
              "   Panesthinae

Order Mantodea
    Family Mantidae
        Subfamily Mantinae
              "   Liturgousinae
              "   Oligonicinae
              "   Photininae

Order Isoptera
    Family Termitidae
        Subfamily Calotermitinae

Order Phasmida
    Family Phasmidae
        Subfamily Bacunculinae
              "   Phibalosominae
              "   Anisomorphinae

Order Orthoptera (Saltatoria)
    Suborder Ensifera
        Superfamily Grylloidea
            Family Gryllotalpidae
                "   Gryllidae
                    Subfamily Oecanthinae
                          "   Trigonidiinae
                          "   Nemobiinae
                          "   Gryllinae
                          "   Myrmecophilinae
                          "   Mogoplistinae
                          "   Eneopterinae
        Superfamily Tettigonioidea
            Family Tettigoniidae
                    Subfamily Phasgonurinae
                          "   Phaneropterinae
                          "   Copiphorinae
                          "   Conocephalinae
                          "   Decticinae
            Family Stenopelmatidae
                    Subfamily Stenopelmatinae
                          "   Henicinae
            Family Rhaphidophoridae
                "   Prophalangopsidae
    Suborder Caelifera
        Superfamily Tridactyloidea
            Family Tridactylidae
                "   Cylindrachaetidae
        Superfamily Acridoidea
            Family Acrididae
                    Subfamily Acridinae
                          "   Oedipodinae
                          "   Romaleinae
                          "   Cyrtacanthacrinae
            Family Tetrigidae (Acrydiidae)
                    Subfamily Tetriginae
                          "   Batrachidinae
Order Grylloblattaria
    Family Grylloblattidae
Order Plecoptera
    Family Perlidae
Order Dermaptera
    Family Labiduridae

Historical Review

Since Linnaeus (80) based his classification of insects upon the structure of the wings, and Fabricius (49) based his upon the structure of the mouth parts, the search for an adequate basis of classification has continued. This has led to extensive comparative studies of the various organ systems of insects. Many authors have made such studies in the Orthoptera and have derived therefrom schemes to show the phylogenetic relationships existing among the various groups.

Bordas (14, 16) studied the structure of the alimentary canal of orthopteroid insects and divided them into two groups, the Acolotasia, with no diverticula or appendages at the mid-gut, and the Colotasia with one or more diverticula at the anterior end of the mid-gut. Later (18) he published an account of a comparative study of the nervous system of representatives of the families of the Orthoptera. He found that in the Blattidae and Mantidae there were unpaired abdominal ganglia and that in the Gryllidae, Tettigoniidae, and Acrididae there were paired abdominal ganglia.

Jensen (73) studied the spermatophores of the Gryllidae and concluded that they are of value in distinguishing the species of *Gryllus* from one another.

The chromosomes of the Tetrigidae and the Acrididae were studied by Robertson (97). He found that these two families had chromosomes of characteristic number, and that the genera of the Tetrigidae could be divided into two groups on the basis of the size of the chromosomes.

Walker (111, 112) investigated the terminal abdominal structures of male and female orthopteroid insects. His conclusions were expressed in a 'phylogenetic tree' in which the blattids, mantids, and termites were closely associated, and the 'Orthoptera' consisted of the long-horned grasshoppers, crickets, sand crickets, and locusts. The Grylloblattoidea and Phasmoidea were shown to be more closely related to the blattoid insects than to the Orthoptera.

Crampton (29, 31, 35) used several external characters in his comparative studies of the Orthoptera. His conclusions were somewhat similar to those of Walker but he considers that the Phasmids and Dermaptera are more closely related to the Orthoptera than to the blattoid insects.

Ford (53) studied the abdominal musculature of the orthopteroid insects. She concluded that "the dispositions of the muscles confirm the relationship of the Blattaria with the Mantaria, Phasmaria and Grylloblattaria, although the latter order resembles considerably the Orthoptera".

Ohmachi (84) divided the Gryllodea into two groups, basing his conclusions upon a study of the chromosomes.

Handlirsch (56, 57) in his exhaustive study of the fossil record divided the orthopteroid insects into two superorders, Orthopteroidea and Blattaeformia, each of which included several orders.

Karny (75) studied the phylogeny of the Ensifera and concluded that the Acridoidea and Grylloidea were derived from ancestors similar to the genus *Gryllacris*.

The external characters of the digestive tracts of species of *Tridactylus*, *Cylindroryctes*, *Acrydium*, and *Gryllotalpa* were investigated by Carpentier (27). He concluded that *Tridactylus* was properly classified with *Cylindroryctes* and *Acrydium* rather than with *Gryllotalpa*.

In his *Anatomie and Phylogenie of the Ensifera*, Ander (1) studied the internal and external anatomy of these insects. His results show that the Ensifera and Caelifera form two distinct groups although they are commonly associated in the order Saltatoria. Zeuner (122) also investigated the Ensifera basing his study upon the fossil record. His conclusions concerning the relationship of the Ensifera to the Acridoidea are similar to those of Ander.

Slifer (102, 103) studied the internal genitalia of female Acrididae and classified the Acrididae on the basis of the structure of the diverticula of the spermathecae and the glandular pouches of the genitalia.

The nervous system of orthopteroid insects was the subject of a comparative study by Nesbitt (83). He divided them into two groups on the basis of the number of posterior recurrent nerves: (i) with one recurrent nerve: Mantidae, Blattidae, Phasmatidae, Grylloblattidae, Isoptera, Dermaptera; (ii) with two recurrent nerves: Tettigoniidae, Rhaphidophoridae, Gryllidae, Acrididae.

Rau (93) discussed the derivation of termites from cockroaches as revealed by study of their method of oviposition and habits.

Roberts (95) drew up a scheme, based primarily upon a study of the phallic structures, to show the relationship of the subfamilies of the Acrididae to one another, and Isely (71) made a comparative study of the mandibles in the same family.

Walker (113, 114, 116, 117) studied the structure of several systems of *Grylloblatta campodeiformis* Walker and emphasized the relationship of the Grylloblattaria with the Saltatoria, especially the Ensifera, rather than with the Blattodea, Mantodea, and Isoptera, the view which he previously expressed (111, 112).

Material and Technical Methods

MATERIAL

The proventriculus in 115 species of insects is studied: Blattodea—12; Mantodea—6; Phasmida—4; Orthoptera (Grylloidea—18; Tettigonioidea—34; Caelifera—37); Grylloblattaria -1; Isoptera—1; Plecoptera—1; Dermaptera—1. The description of the intima of the proventriculus of each species is, in most cases, based upon the study of a single whole mount.

MEASUREMENTS

Measurements of structures were made with the aid of an ocular micrometer in the ocular of a compound microscope. The dimensions of structures in the intact digestive tract and in whole mounts are approximate and are given in centimeters and millimeters. The dimensions of structures in stained microscopic sections are given in millimeters or microns (μ).

Whole Mounts

Whole mounts were prepared to show the sclerotized lining of the proventriculus flattened out and divested of connective tissue and muscles. They were prepared from pinned specimens and specimens preserved in alcohol.

The alimentary canal of a preserved specimen was removed from the body and pinned down, by means of pins through the crop and mid-gut, to a layer of wax in a Syracuse watch glass. The alimentary canal was then covered with water, and was slit longitudinally with a pair of fine eye scissors, this operation being accomplished with the aid of a binocular microscope. The proventriculus was then cut from the alimentary canal, and its muscular coat was removed by careful manipulation with fine needles. In some cases the muscle could not be removed by this method, so the proventriculus was placed for about 12 hr. in a strong solution of potassium hydroxide, which dissolved the muscles.

Pinned specimens were placed in a solution of potassium hydroxide for about 12 hr. The pin was then carefully withdrawn and the dorsal body wall of the insect was cut longitudinally with eye scissors. The sclerotized lining of the proventriculus was removed from the body cavity and was slit longitudinally. Any soft tissues remaining attached were removed with fine needles or by placing the proventriculus in potassium hydroxide for a few hours.

The sclerotized lining of the proventriculus, divested of muscles and other soft tissues, was washed with water to remove the potassium hydroxide. It was then placed in several changes of alcohol of increasing concentrations to remove water, and finally in absolute alcohol. The absolute alcohol was washed out with xylol, and the sclerotized lining was mounted on a slide in Canada balsam or clarite.

Longitudinal Sections and Transverse Sections

The alimentary canals of large newly-killed specimens were removed under water, with the aid of a binocular microscope and were immediately placed in Bouin's fixative. Small, soft insects were carefully opened along the mid-dorsal line and placed in the fixative. The specimens were left in the fixative for from six hours in the case of the smallest insects to 20 hr. in the case of the largest insects. They were then cleared of fixative by repeated changes of 70% alcohol and were stored in 70% alcohol. After being embedded in wax and sectioned at 10μ with a rotary microtome, the tissues were stained with Haidenhain's haematoxylin and counterstained with eosin. By this process nuclei were stained blue, muscles and connective tissue were stained red, while exocuticle remained yellow or brown.

Descriptions of Types

BLATTODEA

BLATTIDAE

Historical Note

Authors who first studied the function of the digestive tract of insects worked on large insects of common occurrence such as the cockroaches. Of these, *Blatta (Periplaneta) orientalis* Linnaeus has received a great deal of attention and consequently the structure of its digestive tract has been thoroughly investigated by several authors: Basch (5), Miall and Denny (81), Visart (110), Bordas (13, 16), Petrunkewitsch (88), Ramme (92), and Eidmann (45).

Cuénot (36) studied the physiology of orthopteroid insects and illustrated his work with a longitudinal section of the digestive tract of *Ectobia perspicillaris* Herbst. (*E. livida* Fabricius). A short description of the proventriculus of *Blattella germanica* Linnaeus was given by Ross (98).

In his comparative study of the digestive tracts of Orthoptera, Bordas (10, 13, 16) investigated a number of species of cockroaches. He described briefly the structure of the proventriculi and arranged the species in four groups in accordance with the complexity of structure of the proventriculus.

Original Descriptions

Pseudomopinae

Parcoblatta pennsylvanica DeGeer

The proventriculus (Fig. 1-P) is conical in shape with the crop (C) leading into the broad end of the cone and the apex of the cone leading into the mid-gut (MG) the anterior end of which bears eight tubular gastric caeca (GC). The proventriculus is 1.5 cm. long. In the anterior region of the proventriculus there are six sclerotized teeth (Fig. 13—CT) projecting into the lumen. Each tooth is flattened laterally. Midway along its length it bears a sharp spine (S), which projects posteriorly. Behind this spine the tooth broadens out slightly and bears much smaller closely-set spines (Ss) projecting posteriorly. Between each pair of teeth there are seven parallel folds projecting slightly into the lumen and bearing fine hairs. The middle, primary fold (PF) is the longest of the seven and extends posteriorly well beyond the posterior limit of the teeth. At either side of the primary fold there are three secondary folds (SF) that are shorter and narrower than the primary folds. Behind each tooth there is a pair of cushions bearing closely-set hairs and projecting into the lumen. The anterior cushion (AC) is 0.3 mm. long and its width is slightly less than its length. The posterior cushion (PC) is 0.2 mm. wide at its anterior end and tapers gradually to join the oesophageal valve (Oes. V.).

Longitudinal section and transverse section (Figs. 14, 15).—The inner layer of the proventriculus is a sclerotized intima (I). On the teeth (CT) it is most heavily developed, particularly in the spine (S) of the tooth where it is 20 μ

in thickness.   On the anterior cushions (AC) it is thin (3 to 4 μ) and bears closely-set hairs (H).   Beneath the intima is a layer of epithelial cells (EP). Throughout the length of the proventriculus this layer is 15 to 30 μ thick and is continuous with the epithelial layer of the oesophageal valve (Oes. V.). Next to the epithelial layer is the longitudinal muscle (LM).   In the anterior region of the proventriculus it is two or three fibers in thickness, while in the teeth it is six to eight fibers in thickness.   Some of these fibers extend obliquely as retractor muscles (RM) and are connected with the epithelium below the intima of the teeth.   Outside the longitudinal muscle is a layer of circular muscle (CM).   This is most strongly developed in the region of the teeth, being six to eight fibers in thickness, and less well developed in the posterior region of the proventriculus, being two to three fibers in thickness.

   Blattella germanica Linnaeus

   Aglaopteryx gemma Hebard

   Ischnoptera deropeltiformis Brunner

   Supella supellectilium Serville

   Cariblatta lutea lutea Saussure and Zehnter

   The proventriculus in these species is similar to that of *Parcoblatta pennsylvanica* except in size.   In *B. germanica* it is 0.5 mm. long; in *A. gemma*, 0.4 mm. long; in *I. deropeltiformis*, 0.9 mm. long; in *C. l. lutea*, 0.4 mm. long; and in *S. supellectilium*, 0.6 mm. long.

Blattinae

   Periplaneta americana Linnaeus

   The proventriculus is conical and 3.5 mm. long.   The anterior half is occupied by six heavily sclerotized teeth (Fig. 18, CT), which are 1.5 mm. long and project into the lumen of the proventriculus.   The base of each tooth is roughly rectangular and 0.5 mm. wide.   The teeth are of such a shape that when the proventriculus is intact they fit snugly together at their tips, leaving six channels between the bases.   Two of these teeth, 1 and 5, are mirror images of one another with a strong projection midway along their length.   Two other teeth, 2 and 4, are mirror images of one another and are grooved laterally.   Tooth 3 has a flat surface toward the lumen of the proventriculus and has a groove at the tip on each side.   In the intact proventriculus it opposes Tooth 6, which has a curved spine at its anterior end and a small groove at each side midway along its length.   Between each pair of teeth there are three parallel folds projecting slightly into the lumen, and bearing fine hairs.   The middle or primary fold (PF) is 1.5 mm. long and the secondary folds (SF) are slightly shorter.   Following each tooth there is a pair of cushions bearing close-set hairs.   The anterior cushion (AC) is 0.5 mm. long and square at its base.   The posterior cushion (PC) is 0.5 mm. broad anteriorly and 1 mm. long and tapers gradually to a point where it reaches the oesophageal valve (Oes. V.).   Between the anterior ends of each adjacent pair of posterior cushions there is a small secondary cushion bearing hairs (SC).

Eurycotis floridana Walker

In this species the proventriculus is similar in all respects to that of *Periplaneta americana* except that it is slightly larger, being 4 mm. long.

Blatta orientalis Linnaeus

In this species the proventriculus is similar in all respects to that of *Periplaneta americana* except that it is considerably smaller, being 2 mm. long.

Panchlorinae

Pycnoscelus surinamensis Linnaeus

The proventriculus is conical and 1 mm. long. In the anterior end there are six small teeth (Fig. 16—CT). They are about 100 μ long and slightly less in width. At its base and toward its posterior end each tooth bears small spines, and projecting into the lumen of the proventriculus there is a large spine (S). Each tooth is situated on the anterior end of an ovoid patch (OP) bearing closely-set bristles. This patch is 0.5 mm. long and tapers posteriorly to a point. Between each pair of these patches there is a single primary fold (PF) similar in shape to the ovoid patches, but shorter. Fol'owing each of the ovoid patches, there is a cushion (C) 0.05 mm. in width and extending to the oesophageal valve (Oes. V.).

Blaberinae

Blaberus atropos Stoll

The proventriculus is conical and 3 mm. long. In the anterior end are six teeth (Fig. 17—CT). The base of each tooth is roughly square and 0.2 mm. long. The tooth projects posteriorly in a broad spine and bears a few irregularly placed smaller spines. Beneath the spine the posterior surface of the tooth is concave. Each tooth is situated on a square patch covered with fine bristles (P). Between the patches there are six primary folds (PF) 1 mm. long and covered with fine bristles. Behind each patch are two cushions covered with bristles. The anterior cushion (AC) is 1 mm. long and 0.5 mm. wide. The posterior cushion (PC) is 1.5 mm. long and 0.5 mm. wide and tapers slightly toward the oesophageal valve (Oes. V.).

Panesthinae

Cryptocercus punctulatus Scudder

The proventriculus is conical and 2 mm. long. In the anterior end are six teeth (Fig. 19—CT) 0.5 mm. in length. The base of each tooth is rectangular and about 0.2 mm. wide. The heavily sclerotized portion of the tooth is a laterally flattened projection rising to a peak near the anterior end of the tooth. Between each pair of teeth there are seven folds, each equal in length to the teeth. The three primary folds (PF) are 0.1 mm. wide and the four secondary folds (SF) are 0.05 mm. wide. All seven are sclerotized and bear small overlapping spines. Behind each tooth there are two cushions. The anterior cushion (AC) projects into the lumen and bears two spines; the anterior spine (AS) is the larger and is surrounded by small spines at its base and over the surface of the cushion; the posterior spine (PS) is smaller

and is covered with smaller scalelike spines. The posterior cushion (PC) bears a spine similar to the posterior spine of the anterior cushion, and tapers gradually toward the posterior where it joins the oesophageal valve (Oes. V.).

MANTODEA

MANTIDAE

Historical Note

The structure of the proventriculus of the common mantid *Mantis religiosa* Linnaeus has been described by Visart (110), Bordas (16), and Ramme (92). Bordas also studied the digestive tract of several other species of Mantidae and described the proventriculus in *Tenodera australasiae* Leach, *Hierodula viridis* Forskal (*H. bioculata* Burmeister), *Stagmatoptera predicatoria* Stoll, *S. annulata* Stoll, and *Eremiaphila denticollis* Lefebr.

Original Descriptions

Mantinae

Mantis religiosa Linnaeus

In this species the proventriculus is conical and 2 mm. long (Fig. 2—P). Its broad end opens from the crop. Just posterior to its point of union with the mid-gut (MG) there are eight tubular gastric caeca (GC). On the inner surface anteriorly there are six areas of narrow longitudinal ridges (Fig. 20—AR). On each of these areas the ridges anastomose posteriorly toward a point midway in the length of the proventriculus where they end at a short cluster of hairs (CC). In transverse section (Fig. 22) the ridges are seen to be narrow and forked at their tips (FT) owing to the presence of grooves that run throughout the length of each ridge. Alternating with the areas of anastomosing ridges are six teeth (Fig. 20—CT) that run the length of the anterior half of the proventriculus. (Four of these are shown in the figure.) Directly behind each tooth there are two cushions. The anterior cushion (AC) is densely clothed with hairs and posteriorly may break up into three to five folds likewise clothed with hairs. The posterior cushion (PC) is elongated and clothed with hairs and extends posteriorly to the oesophageal valve (Oes. V.).

Transverse sections (through region of teeth—Fig. 21; through anterior cushions—Fig. 23; through anastomosing ridges—Fig. 22).—The innermost layer of the proventriculus is the intima (I), which is generally 20μ thick, although slightly thicker over the teeth (CT), and appearing as forked projections (FT) in the anastomosing ridges. Over the anterior cushions the intima bears sharp hairs (CH). Beneath the intima is a single layer of epithelial cells (EP), 20 to 25 μ thick. Next to the epithelial layer is the longitudinal muscle (LM), which is several strands in thickness beneath the longitudinal teeth and in the anterior cushions. The outer layer of the proventriculus is circular muscle (CM), four to six fibers thick.

Stagmomantis carolina Johannson

In this species the proventriculus is similar to that of *M. religiosa* but is larger, being 3 mm. long.

Liturgousinae

Gonatista grisea Fabricius

The proventriculus is similar to that of *M. religiosa* but is smaller, being 1.5 mm. long.

Oligonicinae

Oligonicella scudderi Saussure

Thesprotia graminis Scudder

The proventriculus in these two species is similar to that of *M. religiosa* but is much smaller, being 1 mm. long.

Photininae

Brunneria borealis Scudder

The proventriculus is similar in size and structure to that of *M. religiosa*,

Terms Used by Authors in Describing the Proventriculus of Blattodea
and Mantodea

Sclerotized tooth:

—heavily chitinized tooth (Basch)
—anterior tooth (Bordas, Eidmann)
—chitinous tooth (Mantodea) (Bordas)
—tooth (Ross)

Anterior cushion:

—anterior cushion (Basch)
—posterior tooth (Bordas)
—first fold of posterior proventriculus (Eidmann)
—membranous cushion (Ross)

Posterior cushion:

—posterior cushion (Basch)
—muscular fold (Bordas)
—second fold of posterior proventriculus (Eidmann)
—band of muscle (Ross)

Primary fold:

—primary fold (Basch)
—flattened denticle (Bordas)
—principal fold (Eidmann)
—spatulate loop (Ross)

Secondary fold:

—secondary fold (Basch)
.—flattened denticle (Bordas)
—smaller folds (Eidmann)

In Mantodea, anastomosing ridges (Bordas) are present in place of primary and secondary folds in Blattodea.

PHASMIDA

PHASMIDAE

Historical Note

Bordas (12, 16) described briefly the structure of the crop and proventriculus of *Phibalosoma pythonius* Westwood, *Acanthoderus spinosus* Gray, and *Necroscia erectheus* Westwood. Of this part of the fore-gut he says "it can be homologized, from an anatomical point of view, with the gizzard of most Orthoptera".

Heymons (61) described the digestive tract of *Bacillus rossii* Fabricius and says that a "Kau—oder muskelmagen" is altogether absent.

deSinéty (40) described briefly the proventriculus of a species of Phasmidae and Cameron (24) described the proventriculus of *Bacillus rossii* Fabricius.

Original Descriptions

Bacunculinae

Diapheromera femorata Say

In this species the proventricular region is comparatively simple in structure (Fig. 3– P). It is cylindrical and continuous with the crop (C) and enters the mid-gut (MG) as a slender oesophageal valve (Fig. 25—Oes. V.). The inner surface of the crop and proventriculus is occupied by longitudinal folds (LF) 0.2 mm. in width. The surface of each fold is armed with small spines (Fig. 26—S) projecting into the lumen of the proventriculus. On each fold these spines are arranged in transverse rows with six in each row.

Longitudinal section (Fig. 24). –The inner layer of the proventriculus is the intima (I), which is composed of two layers. The innermost layer is 5 to 10 μ thick and bears the small spines. The second layer is 30 to 40 μ thick. Next to the intima is the epithelial layer (EP) of small irregular cells 25 to 50 μ thick. It extends throughout the crop, proventriculus, and oesophageal valve (Oes. V.) and is continuous with the much thicker (60 to 80 μ) epithelial layer of the mid-gut (MG). Outside the epithelial layer is a sparse layer of longitudinal muscle (LM) and outside this a layer of circular muscle (CM) three to five fibers thick. In some specimens the wall of the proventriculus shows a thick invagination (Inv.). Posterior to the proventriculus the oesophageal valve (Oes. V.) projects into the mid-gut as a long flap (Fl.).

Manomera tenuescens Scudder

The proventriculus in this species is similar to that of *D. femorata*.

Phibalosominae

Aplopus mayeri Caudell

The proventriculus is similar to that of *D. femorata*.

Anisomorphinae

   Anisomorpha buprestoides Stoll

     The gross structure of the proventriculus is the same as that of *D. femorata.* The surface of each longitudinal fold (Fig. 27—LF) bears rounded, irregularly-placed scales (S). Some of these are simple and others are larger and bear other smaller rounded scales.

     Terms Used by Authors in Describing the Proventriculus of Phasmidae

Longitudinal folds:

    —striated folds (Bordas)

    —longitudinal folds (Bordas)

Small spines:

    —small spines (deSinéty)

    —chitinous teeth (Bordas)

ORTHOPTERA

Ensifera

GRYLLOIDEA

Historical Note

     The digestive tract of the European mole cricket, *Gryllotalpa vulgaris* Latreille, has been studied by several investigators and its proventriculus has been described by Wilde (119), Eberli (44), Cuénot (36), Bordas (16), and Ramme (92). The proventriculus of *Gryllotalpa australis* Erichson was described by Sayce (99), and Carpentier (27) noted the external features of the proventriculus of *Gryllotalpa gryllotalpa* Linnaeus.

     The structure of the proventriculus of the field cricket, *Gryllus campestris* Linnaeus, was described by Graber (54) and by Berlese (6) who called it a "ventrilio". Graber also studied the organ in *Gryllus melas* and *Oecanthus pellucidus.* Wilde (119) described the proventriculus of the house-cricket, *Gryllulus domesticus* Linnaeus. Other members of the Gryllinae in which the proventriculus has been described are *Gryllulus pennsylvanicus* Burmeister (DuPorte (43)) and the Chinese cricket, *Gryllus mitratus* Burmeister (Hsu (69)), and in the Myrmecophilinae, a family of crickets inhabiting ant hills, the structure of the proventriculus of *Myrmecophila acervorum* Latreille has been investigated by Schimmer (100).

     Bordas (11, 13, 18) described the proventriculus of several crickets and divided them into three groups on the basis of complexity of structure.

Original Descriptions

GRYLLIDAE

Oecanthinae

   Oecanthus nigricornis Walker

     The proventriculus consists of a globular body (Fig. 4—P) joined to the crop (C) by a tubular neck. It lies between the two bulbous gastric caeca

(GC) of the mid-gut (MG).   The lining of the proventriculus is organized into six longitudinal folds.   In the neck each of these folds bears a series of lobes covered with hairs (Fig. 32—CL).   In the globular part of the organ each fold bears a series of nine sclerotized appendages (CA).   The anterior appendage in each series is 0.3 mm. wide and the size of the others decreases gradually toward the posterior appendage, which is 0.15 mm. wide.   The central portion of each appendage is a median tooth (Fig. 40—MT), which projects into the lumen of the proventriculus and bears five or six pointed median denticles (MD).   From the center of the base of the median tooth there is a pair of lateral teeth (LT) projecting posteriorly and bearing several short spines that project laterally.   From the side of the median tooth and below the lateral tooth a lateral denticle (LD) extends outward and bears a few rounded lobes.   Beneath these teeth and denticles there is a truncated lobe, the inner barbated lobe (IBL), which extends laterally.   It is less heavily sclerotized than the other parts and bears fine bristles at its outer extremity.   At each side of the appendage there is an outer barbated lobe. This has a roughly square base and tapers, toward the lumen of the proven-triculus, to a blunt point (Fig. 32—OBL).   Posterior to the last appendage in each longitudinal fold the oesophageal valve extends into the mid-gut and ends as a round flap (Oes. V.).   Between adjacent longitudinal folds there is a partition (CP) coextensive with the series of appendages.

Transverse sections (through median tooth, Fig. 29; through median denticles, Fig. 30; through lateral teeth, Fig. 31).—The inner layer of the proventriculus is the intima (I).   This is thickest where the teeth and denticles have lobes and spines as in the lateral teeth (LT) and lateral denticles (LD).   Beneath the intima is the epithelial layer (EP) and beneath this the longitudinal muscle (LM), one to four fibers thick.   Outside the longitudinal muscle is the circular muscle (CM), six to seven fibers thick.

Neoxabea bipunctata DeGeer

In this species the proventriculus is of the same size and structure as that of *O. nigricornis*.

Trigonidiinae

Falcicula hebardi Rehn

In this species each longitudinal fold in the globular part of the proven-triculus is 0.3 mm. long and 0.2 mm. wide (Fig. 33).   Each fold bears four appendages.   The median tooth (MT) is bifurcated and bears a few irregular median denticles (MD).   The lateral tooth (LT) extends from the central part of the median tooth laterally and then posteriorly to well beyond the posterior extremity of the median tooth.   It bears sharp spines projecting posteriorly.   The inner barbated lobe (IBL) is rounded, and the outer bar-bated lobe (OBL) bears short, blunt spines.   The partition (CP) is co-extensive with the series of four appendages.

Anaxipha exigua Say

The globular part of the proventriculus is 0.5 mm. long and each longitudinal fold is 0.25 mm. wide and bears six appendages, the structure of which is the same as in *Falcicula hebardi*.

Phyllopalpus pulchellus Uhler

The globular part of the proventriculus is 0.6 mm. long and each longitudinal fold bears nine appendages, the structure of which is the same as in *Falcicula hebardi*, except that the posterior branches of the lateral teeth (Fig. 34—LT) do not extend beyond the median tooth.

Cyrtoxipha columbiana Caudell

The globular part of the proventriculus is 0.5 mm. long and each longitudinal fold bears six appendages. The structure of these is the same as in *Falcicula hebardi* except that the lateral teeth (Fig. 35—LT) do not extend beyond the median tooth.

Eneopterinae

Hapithus brevipennis Saussure

The proventriculus is 5 mm. long. Each of the six longitudinal folds in the globular part is 2.5 mm. long and has 12 to 13 appendages. The anterior appendage on each fold is 0.6 mm. wide and the others are progressively smaller toward the last one, which is 0.3 mm. wide. On each appendage the median tooth (Fig. 42—MT) has a long posterior projection bearing six to eight median denticles (MD). The lateral denticles (LD) are short and have a few short serrations. The lateral teeth (LT) extend posteriorly slightly beyond the extremities of the lateral denticles. The inner barbated lobe (IBL) is rounded and bears hairs, and the outer barbated lobe (OBL) has a flat surface toward the lumen of the proventriculus.

Orocharis saltator Uhler

The proventriculus is similar in form to that of *Hapithus brevipennis* but is slightly less than one half its size.

Tafalisca lurida F. Walker

The proventriculus is 3 mm. long and each fold in the globular part is 1.5 mm. long. The form of the appendages is the same as in *H. brevipennis* and *O. saltator* except that the posterior projection of the median tooth (Fig. 43—MT) is only half as long as in these two species.

Nemobiinae

Nemobius fasciatus DeGeer

The proventriculus is 3 mm. long and each longitudinal fold in the globular part is 1 mm. long. Some of the lobes in the neck bear a few spines projecting posteriorly. Each longitudinal fold in the globular part bears nine appendages. The anterior appendage on each fold is 0.3 mm. wide and the others are progessively smaller toward the posterior one, which is 0.15 mm. wide. In each appendage the median tooth (Fig. 38—MT) has a posterior

projection with roughly parallel borders bearing three or four short median denticles (MD). The lateral teeth (LT) have rounded lobes. The lateral denticles (LD) are truncated and concave at their extremities. The inner barbated lobes (IBL) are rounded and beset with hairs.

Gryllinae

Gryllulus domesticus Linnaeus

The proventriculus is 4 mm. long. In the neck some of the lobes bear a few spines projecting posteriorly. Each longitudinal fold in the globular part has 11 appendages, the anterior one being 0.5 mm. wide and the posterior one 0.25 mm. wide. The median tooth (Fig. 39—MT) has a posterior projection with six rounded median denticles (MD). Each lateral denticle consists of a single rounded lobe (LD). The lateral tooth (LT) has several rounded projections. The inner barbated lobe (IBL) is broad and has bristles along its posterior border.

Gryllulus assimilis Fabricius

The proventriculus in this species is similar in size and structure to that of *Gryllulus domesticus.*

A longitudinal section of a short portion of the neck and the globular part of the proventriculus is shown in Fig. 36. It is taken through the mid-line of the median teeth (MT) of opposing longitudinal folds. The inner layer is composed of intima (I) which is thinnest (30 to 50 μ) in the neck (N) and thickest (50 to 75 μ) on the median denticles (MD). Beneath the intima is the epithelial layer (EP) composed of small, roughly cubical cells. This layer projects into the hollows of the appendages and is continuous with the epithelial layer of the gastric caeca (GC) of the mid-gut. Next to the epithelium is the longitudinal muscle (LM). This is most extensive in the appendages where it runs obliquely between the epithelium and the circular muscle. The circular muscle (CM) is six to seven fibers in width at the anterior and posterior ends of the proventriculus and 10 to 12 fibers at the widest part of the proventriculus. The oesophageal valve (Oes. V.) constricts the lumen of the proventriculus at its posterior end and projects into the mid-gut between the gastric caeca.

Miogryllus verticalis Serville

In this species the structure of the proventriculus is similar to that of *G. assimilis* and *G. domesticus* but it is shorter. The length of the whole proventriculus is 2 mm., the length of each longitudinal fold in the globular part is 1 mm., and the number of appendages in each fold is nine.

Anurogryllus muticus DeGeer

The proventriculus is 3.5 mm. long and each longitudinal fold is 2 mm. long and bears 13 appendages, the anterior one of which is 0.5 mm. wide and the posterior 0.25 mm. The form of each appendage differs considerably from the similar structure in *G. assimilis* and *G. domesticus.* The median tooth (Fig. 47—MT) extends posteriorly in a long projection that bears

10 or 12 sharp median denticles (MD). The lateral denticles (LD) and lateral teeth (LT) have several rounded spines at their outer borders. The inner barbated lobes (IBL) extend posteriorly beyond the lateral denticles and bear bristles.

Mogoplistinae

Cycloptilum squamosum Scudder

The proventriculus is 1.5 mm. long and each longitudinal fold is 0.7 mm. long and bears eight appendages. The median tooth (Fig. 44—MT) has a short projection posteriorly with three to five median denticles (MD). The lateral denticles (LD) are short and have several rounded spines. The lateral teeth (LT) project beyond the lateral denticles and have several rounded spines. The inner barbated lobes (IBL) are rounded and bear bristles. The outer barbated lobes (OBL) are roughly triangular. At either side of a longitudinal fold is a partition (CP) that is slightly swollen at its posterior end and has short, scalelike projections. At the posterior end of each longitudinal fold is the round flap of the oesophageal valve (Oes. V.), which bears bristles at its anterior end.

Myrmecophilinae

Myrmecophila oregonensis Brunner

This species has the smallest proventriculus of any studied in the Gryllidae. Its total length is 1 mm. and the length of the longitudinal folds in the globular part is 0.25 mm. Each fold bears five appendages, the first of which is 0.1 mm. wide and the last 0.05 mm. wide. Each appendage is less complicated than are comparable structures in other Gryllidae studied. There is no posterior projection of the median tooth (Fig. 45—MT). The lateral denticles (LD) are represented by small lobes, and the lateral teeth by a series of blunt projections at each side of the appendage (LT). There are no inner barbated lobes. The outer barbated lobes (OBL) are irregular in shape and are progressively smaller toward the posterior lobe. Following the longitudinal fold is the flap of the oesophageal valve (Oes. V.). At either side of each longitudinal fold and coextensive with it is a partition (CP) that continues further parallel with the oesophageal valve. It bears small scales in the region of the anterior part of the oesophageal valve.

GRYLLOTALPIDAE

Scapteriscus vicinus Scudder

The proventriculus is 5 mm. long and each longitudinal fold in the globular part of the proventriculus is 2 mm. long. Between the neck and the globular part on each longitudinal fold there is a cushion (Fig. 41—CC) bearing hairs. Each longitudinal fold bears 12 to 13 appendages. The anterior one is 0.5 mm. wide and the posterior one 0.25 mm. wide. The median tooth has no posterior projection but bears a row of blunt median denticles (Fig. 37—MD). The lateral denticles (LD) are truncated and concave at their margins. The lateral teeth (LT) are broad at the tip. The

inner barbated lobes (IBL) are pointed and beset with bristles.   The outer barbated lobes (Fig. 41—OBL) are roughly square at the base and extend 'rregular projections into the lumen.   The partitions (CP) are parallel to the longitudinal folds and have small scalelike projections.

Gryllotalpa hexadactyla Perty

The proventriculus is 6 mm. long and each longitudinal fold in the globular part is 3 mm. long and bears 14 appendages.   The structure of these appendages is the same as in *S. vicinus* except that there is only a single median denticle (Fig. 47—MD) in the form of a short tubercle.

Terms Used by Authors in Describing the Proventriculus of Grylloidea

Median tooth:
—small median tooth (Berlese)
—median point of median tooth (Bordas)
—median tooth (DuPorte)

Median denticles:
—median denticles (DuPorte)

Lateral tooth:
—small internal tooth (Berlese)
—lateral points of median tooth (Bordas)
—lateral tooth (DuPorte)

Lateral denticles:
—lateral points of median tooth (Bordas)
—lateral denticles of median tooth (DuPorte)

Inner barbated lobe:
—small external tooth (Berlese)
—lateral lobe of median tooth (Bordas)
—inner barbated lobe (DuPorte)

Outer barbated lobe:
—lateral tooth (Bordas)
—outer barbated lobe (DuPorte)

Sclerotized partition:
—chitinous line (Berlese)
—chitinous partition (Bordas, DuPorte)

TETTIGONIOIDEA

Historical Note

Mulder (82) figured a lateral view of the digestive tract of a "Locustid" but gave no details of the internal structure of the proventriculus.

The structure of the proventriculus of the common European insect *Tetti-gonia verrucivora* Linnaeus (*Decticus verrucivoris* Linnaeus) has been described

by Graber (54) and Wilde (119). The structure of the proventriculus of *Phasgonura viridissima* (*Locusta viridissima*) Linnaeus has been investigated by Wilde (119) and Ramme (92). Other European species in which the proventriculi have been studied are *Conocephalus fuscus* (Fabricius) = *Xyphidium fusc m* Fabricius (Graber, 54), *Ephippiger ephippiger* Fiebig (*E. vitium* Serville) (Graber, 54), and *Meconema varia* Fabricius (Wilde, 119).

Bordas (16) described briefly the proventriculus of several species of the "Locustidae" in his comparative studies of the digestive tract of orthopteroid insects. Davis (37) investigated the structure of the proventriculus of the large tettigoniid *Stenopelmatus fuscus* Haldemann.

Snodgrass (104) studied the external features of the digestive tract of *Peranabrus scabricollis* (Thomas) but gave no details on the internal structure of the proventriculus.

Original Descriptions

Tettigoniidae

Copiphorinae

Neoconocephalus ensiger Harris

The body of the globular part of the proventriculus is 2 mm. long (Fig. 5—P) and it is joined to the crop (C) by a tubular neck (N). Where it joins the mid-gut (MG) it is embedded between two bulbous gastric caeca (GC). In the neck there are six longitudinal rows of cushions covered with hairs (Fig. 48—C) with 8 to 12 cushions in each row. The anterior cushions are small and bear few hairs while the posterior cushions are broad and bear a heavy coat of hairs. All but the last four cushions in each row bear a pointed projection (CT) that extends posteriorly and has sharp spines on its surface. Between each two rows of cushions in the neck there is a series of folds in the form of a double loop (CH) covered with hairs. Each of the six longitudinal folds in the globular part of the proventriculus has 12 appendages (T). The anterior one is 0.8 mm. wide and the size of the others decreases regularly toward the posterior one, which is 0.3 mm. wide. Each appendage has a median tooth (MT), which projects into the lumen of the proventriculus and has a serrated posterior border. From the side of the median tooth there extends to each side a lateral lobe (LL). At each side of the appendage there is a barbated lobe (BL) roughly triangular at the base and with a blunt point projecting posteriorly. Between the longitudinal folds and coextensive with them there is a partition (CP), the surface of which is covered with scale-like projections. At the posterior end of each longitudinal fold there is a flap of the oesophageal valve (Oes. V.) roughly triangular and beset with hairs at its anterior end.

Transverse section (through appendages Fig. 49).—The inner layer is the intima (I), which is thickest (30 to 40 μ) over the median teeth (MT) and the lateral lobes (LL). Beneath this is the epithelial layer (EP), which is composed of rounded or cubical cells and extends into the cavities of the median teeth, barbated lobes (BL), and partitions (CP). The longitudinal muscle

(LM) is one to three fibers thick and the circular muscle (CM) 10 to 12 fibers thick.

Belocephalus subapterus subapterus Scudder

Bucrates malivolans Scudder

Pyrgocorypha uncinata Harris

The proventriculus of these species is the same in size and structure as that of *N. ensiger*.

Conocephalinae

Conocephalus fasciatus DeGeer

The proventriculus is 2 mm. long and each longitudinal fold in the globular part of the proventriculus is 0.8 mm. long and bears nine appendages (Fig. 50—T). The structure of the proventriculus is similar to that of *N. ensiger*.

Transverse sections (Fig. 51—through the neck of the proventriculus; Fig. 53—through a median tooth; Fig. 52—through the oesophageal valve).— The intima (I) is thinnest (5 to 10 μ) in the neck and oesophageal valve and thickest (25 to 30 μ) on the median teeth (MT) and lateral lobes (LL). The epithelial layer (EP) is one cell thick in the neck and oesophageal valve and two to three cells thick in the cavities of the median teeth and barbated lobes (BL). The longitudinal muscle (LM) is one to three fibers thick and the circular muscle four to five fibers thick in the neck and oesophageal valve and six to eight in the globular part of the proventriculus.

Orchelimum gladiator Brunner

The proventriculus is similar in structure to that of *C. fasciatus* but is slightly larger. Its total length is 2.5 mm. and each longitudinal fold bears 11 appendages.

Odontoxiphidium apterum Morse

The proventriculus is similar in size and structure to that of *O. gladiator*.

Phasgonurinae

Phasgonura cantans Fuessly

The proventriculus is similar in size and structure to that of *Neoconocephalus ensiger*.

Decticinae

Atlanticus gibbosus Scudder

In this species the proventriculus has the same general structure as in the Copiphorinae, Conocephalinae, and Phasgonurinae. It is 5 mm. long and each longitudinal fold in the globular part is 2 mm. long. In the neck are the cushions (Fig. 54—C) bearing hairs, the serrated projections (CT), and the loop of hairs (CH). Each longitudinal fold has 18 appendages. The anterior appendage is 0.5 mm. wide and has a heavy armature, and the size of the other appendages decreases regularly toward the posterior one, which is 0.3 mm. wide and has a much weaker armature. The surface of an appendage

is covered with short spines (Fig. 55). The median tooth (MT) projects posteriorly and a shorter lateral projection (LP) extends laterally at each side. There is a smooth lateral lobe (LL) extending from each side of the base of the appendage. At each side of an appendage is the barbated lobe (Fig. 54—BL). The sclerotized partitions (CP) extend parallel to the longitudinal folds and are 0.1 mm. wide. A flap of the oesophageal valve (Oes. V.) extends posteriorly from the end of each longitudinal fold.

Pholidoptera griseoaptera DeGeer

The proventriculus is similar in structure to that of *A. gibbosus* except that the spines covering the appendages are rounded rather than sharp (Fig. 56). The organ is 2.5 mm. long and each longitudinal fold in the globular part is 1.5 mm. long and bears 14 appendages.

Chelidoptera albopunctata Goeze

The proventriculus is 2.5 mm. long and each longitudinal fold is 1.5 mm. long and bears 14 appendages. The structure is the same as in *A. gibbosus* except that the spines on the surface of the appendages are two or three times larger (Fig. 57).

Eremopedes balli Caudell

The proventriculus is the same in all respects to that of *C. albopunctata* except that it is slightly larger, its total length being 3 mm., and the length of each longitudinal fold being 2 mm.

Neduba carinata Walker

The proventriculus is 3 mm. long and each longitudinal fold is 1.5 mm. long. Its structure is the same as in *A. gibbosus* except in the form of the appendages in which the median tooth (Fig. 58—MT) extends backward as a blunt spine bearing irregular tubercles. The lateral projections (LP) are blunt and rounded and the lateral lobes are pointed (LL).

Anabrus simplex Haldemann

The proventriculus is 5 mm. long and each longitudinal fold is 2.5 mm. long and bears 15 appendages in which the median teeth (Fig. 59—MT) and the spines on the surface are rounded, short, and scalelike.

Pediodectes haldemanni Girard

This species has the largest proventriculus of all the Decticinae studied. It is 6 mm. long and each longitudinal fold is 3 mm. long and bears 18 appendages. The surface of each appendage is covered with coarse, sharply pointed spines of irregular length (Fig. 60).

Phaneropterinae

Scudderia curvicauda DeGeer

The proventriculus in this species is similar to that of other species in the Tettigoniidae except that its inner lining has a much less robust armature. It is 3 mm. long and each longitudinal fold in the globular part is 1.5 mm. long. In the neck are the longitudinal rows of cushions bearing hairs (Fig. 69—CH)

and the lobes (CT) are serrated at their tips.   On each longitudinal fold of the globular part there are 18 appendages.   Each appendage has a median tooth (MT) with a serrated posterior border, and a narrow lateral lobe (LL) projecting laterally from each side of the appendage.   The whole surface of the appendage except the median tooth is thickly clothed with hairs and fine spines (CS).   In the posterior six or seven appendages the median tooth is replaced by a tuft of hairs.   The barbated lobes (BL) at each side of each appendage have a small spine surrounded by a tuft of hairs.   The partitions (CP) run parallel to the longitudinal fold at the end of which is the round flap of the oesophageal valve (Oes. V.).

Transverse sections (through posterior part of neck—Fig. 67, through median tooth of appendage—Fig. 68).—The intima (I) is 20 to 25 μ thick over the lateral lobes (LL) and partition (CP) and 5 to 10 μ thick over the remaining surfaces such as the barbated lobes (BL).   The epithelial layer (EP) is one cell in width at the base of the appendages and partitions and three to four cells wide in the cavities of the appendages.   The longitudinal muscle (LM) is one fiber in thickness or absent entirely at the base of the appendages and is three to·four fibers thick in the appendages.   The circular muscle (CM) is six to eight fibers wide.

Arethaea arachnopyga Rehn and Hebard

The structure of the proventriculus is the same as in *Scudderia curvicauda*. It is 2 mm. long and each longitudinal fold in the globular part is 1 mm. long and has 12 appendages.

Montezumina modesta Brunner

The proventriculus is similar in size and structure to that of *Scudderia curvicauda*.

Amblycorypha oblongifolia DeGeer

The proventriculus is similar in size and structure to that of *Scudderia curvicauda*.

Microcentrum rhombifolium Saussure

The proventriculus is similar in size and structure to that of *Scudderia curvicauda*.

Stilpnochlora couloniana Saussure

The proventriculus is similar in structure to that of *Scudderia curvicauda* but is slightly larger, being 4 mm. long.

Insara elegans Scudder

The proventriculus is similar in structure to that of *Scudderia curvicauda* but is only 2 mm. long and has 12 to 13 appendages in each longitudinal fold.

Inscudderia walkeri walkeri Hebard

The proventriculus is similar in size and structure to that of *Scudderia curvicauda*.

STENOPELMATIDAE

Stenopelmatinae

Stenopelmatus fuscus Haldemann

The proventriculus is 3.5 mm. long and each longitudinal fold in the globular part is 1.5 mm. long and bears 11 appendages. It is similar in structure to that in *Neoconocephalus ensiger* Harris but differs slightly in that the median tooth of each appendage is slightly more prominent (Fig. 61—MT).

Henicinae

Hemideina megacephala Buller

The proventriculus is 6 mm. long and each longitudinal fold in the globular part is 4 mm. long and bears 20 to 22 appendages each of which is 0.75 mm. wide. The general structure is similar to that of *Stenopelmatus fuscus* Haldemann but the appendages differ considerably. The median tooth (Fig. 62—MT) has a posterior projection bifurcated at the tip and has small scalelike protuberances on its surface. The lateral lobes (LL) are broad at the tip. The anterior border of the appendage bears hairs.

Cratomelus sp.

The proventriculus is 4 mm. long and each longitudinal fold in the globular part is 2 mm. long and bears 12 appendages. The general structure is similar to that of *Hemideina megacephala* Buller but the appendages are different. The median tooth (Fig. 63—MT) extends posteriorly as a rounded lobe that has a blunt median tip. The lateral lobes (LL) are broad at the tip, and the anterior border of the appendage bears hairs.

PROPHALANGOPSIDAE

Cyphoderris monstrosa Uhler

The proventriculus is 5 mm. long and each longitudinal fold is 2.5 mm. long and bears 12 appendages. The general structure of the proventriculus is the same as in the Copiphorinae and Stenopelmatinae. The median tooth, however, is relatively longer and bears irregular tubercles (Figs. 73, 74—MT). The lateral lobes (LL) are blunt and the surface of the appendage is covered with scalelike protuberances, and the posterior border of the appendage bears hairs.

RHAPHIDOPHORIDAE

Ceuthophilus maculatus Harris

The proventriculus is 3 mm. long and globular (Fig. 6—P) with a neck (N) attached to the posterior end of the crop (C). Where it joins the mid-gut (MG) it is embedded between two bulbous gastric caeca (GC). In the neck are the six longitudinal rows of six to seven cushions (Fig. 66—CC) bearing hairs. Each of the anterior five of these cushions has a roughly rectangular projection (CT) with short spines. Each of the longitudinal folds in the

globular part has 10 or 11 appendages. The median tooth (MT) of each appendage is a tuft of hairs, and a pair of lateral lobes extends outward from the median tooth. The anterior barbated lobes (BL) are roughly square at the base and have a blunt projection toward the lumen of the proventriculus. The posterior barbated lobes are smaller than the anterior and are irregular in shape. Each longitudinal fold is followed by a flap of the oesophageal valve (Oes. V.) and flanked by a partition covered with small scalelike lobes (CP).

Pristoceuthophilus pacificus Thomas

The proventriculus is 2 mm. long and each longitudinal fold in the globular part bears six appendages. The structure is the same as in *Ceuthophilus maculatus* Harris.

Udeopsylla robusta Haldemann

The proventriculus is the same in size and structure as that of *Ceuthophilus maculatus* Harris.

Styracosceles neomexicanus Scudder

The proventriculus is the same in size and structure as that of *Ceuthophilus maculatus* Harris.

Daihinia brevipes Haldemann

The proventriculus is the same in size and structure as that of *Ceuthophilus maculatus* Harris.

Hadenoecus puteanus Scudder

The proventriculus is 3 mm. long and each longitudinal fold is 1.5 mm. long. In the neck there are the cushions (Fig. 28—CC) with their sclerotized lobes (CT). The structure of the appendages in the globular part differs considerably from that in the other Rhapidophoridae studied. The appendages in each longitudinal row are 12 in number, the anterior one being 0.4 mm. wide and the posterior one 0.2 mm. wide. The anterior barbated lobes (BL) are roughly square at the base and bear strong projections extending posteriorly while the posterior ones are much smaller than the anterior and do not bear projections. The partitions (CP) are 0.1 mm. wide anteriorly and taper to a point posteriorly. On an appendage (Fig. 64) there is a blunt median tooth (MT) with short spines. The lateral lobes are short and thick (LL) and the anterior border of each appendage is concave and bears hairs that extend over the appendage toward the lateral lobes.

Tachycines asynamorus Adelung

The proventriculus is similar in size and structure to that of *Hadenoecus puteanus* Scudder except in the structure of the appendages in which the lateral lobes (LL) are subdivided into two smaller lobes (Fig. 65).

Terms Used by Authors in Describing the Proventriculus of Tettigonioidea

Median tooth:
>   —median tooth (Bordas)
>   —chitinous tooth (Davis)

Posterior projection of median tooth:
>   —median lobe (Bordas)
>   —apical denticle (Davis)

Lateral projection:
>   —lateral denticle (Bordas, Davis)
>   —lateral tubercle (Bordas)

Lateral lobe:
>   —lateral lobe (Bordas)

Barbated lobe:
>   —lateral tooth (Bordas)
>   —chitinous tubercle (Bordas)
>   —barbated lobe (Davis)

Sclerotized partition:
>   —chitinous partition (Bordas, Davis)

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CAELIFERA

ACRIDOIDEA

ACRIDIDAE

Historical Note
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Dufour (42) described the proventricular region of *Oedipoda caerulescens*, but regarded the Acrididae as having no "gésier".

Faussek (50) gave a short description of the proventriculus of *Eremobia muricata* Pall.

Visart (110) gave a short description of the proventriculus of *Oedipoda caerulescens* Linnaeus.

Bordas (15) investigated the digestive tract of *Pamphagus elephas* Stal and (16) described briefly the proventricular region of several species of Acrididae.

Tietz (109) described the digestive tract of *Dissosteira carolina* Linnaeus.

Hodge (66, 67, 68) made a detailed investigation of the digestive tract of *Melanoplus differentialis* Thomas, *Locusta migratoria* Linnaeus, and *Radenotatum carinatum* var. *peninsulare* Rehn and Hebard.

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Original Descriptions
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Oedipodinae

Dissosteira carolina Linnaeus

With the crop (Fig. 7—C) and the mid-gut (MG) the proventriculus (P) forms a tube 1 mm. in diameter. At the level at which the proventriculus

joins the mid-gut there are six gastric caeca (GC) of the mid-gut. Each of these has a tubular anterior portion 4 mm. long and a tubular posterior portion 2 mm. long. The lining of the posterior part of the crop shows about 40 longitudinal ridges (Fig. 70—LR) each of which is 0.1 mm. wide and bears a series of flat scalelike projections (S). Posteriorly the longitudinal ridges converge to form six longitudinal plates (LP) of the proventriculus. Each of these is 1 mm. long and 0.5 mm. broad at its anterior end and tapers slightly toward its rounded posterior border. The surface of each plate bears fine scalelike projections. Between the longitudinal plates there are a few weak irregular folds.

Transverse sections (Fig. 75—through posterior end of crop; Fig. 76—through converging longitudinal ridges; Fig. 77—through anterior ends of the longitudinal plates; Fig. 78—through posterior ends of the longitudinal plates).— The inner layer is the intima (I), 20 to 30 μ thick. The epithelial layer (EP) is one cell in thickness. The longitudinal muscle (LM) is two to four strands thick in the longitudinal plates and is associated with a few transverse muscles (TM). The circular muscle (CM) is three to six fibers thick.

> *Arphia sulphurea* Fabricius
> *Arphia pseudonietana* Thomas
> *Encoptolophus sordidus* Burmeister
> *Pardalophora apiculata* Harris
> *Hippiscus rugosus* Scudder
> *Spharagemon bolli* Scudder
> *Scirtetica marmorata* Harris
> *Psinidia fenestralis* Serville
> *Trimerotropis pallidipennis* Burmeister
> *Circotettix verruculatus* Kirby

In these species the proventriculus is similar in structure to that of *Dissosteira carolina* Linnaeus.

Cyrtacanthacrinae

> *Dendrotettix quercus* Packard

In this species the proventriculus is similar to that of *Dissosteira carolina* Linnaeus, having the longitudinal ridges (Fig. 71—LR) and scalelike projections (S) in the crop, and the longitudinal plates (LP) of the proventriculus. The lining is, however, more robust, with stronger scalelike projections in the crop and relatively more scalelike projections on the longitudinal plates of the proventriculus.

> *Gymnoscirtetes pusillus* Scudder
> *Campylacantha olivacea olivacea* Scudder
> *Hesperotettix festirus* Scudder
> *Podisma pedestris* Linnaeus
> *Aptenopedes sphenarioides apalachee* Hebard
> *Paroxya clavuliger* Serville
> *Melanoplus bivittatus* Say

Melanoplus punctulatus Scudder

Melanoplus femur-rubrum DeGeer

Schistocerca americana Drury

The proventriculus in these species is similar to that of *Dendrotettix quercus* Packard and of *Dissosteira carolina* Linnaeus, some being relatively more robust as in the former and some less robust as in the latter, depending upon the size.

Romaleinae

Romalea microptera Beauvais

The proventriculus is similar in structure to that of *Dendrotettix quercus* Packard but in keeping with the size of the insect it is twice as big, the longitudinal plates being 4 mm. long.

Acridinae

Chloealtis conspersa Harris

The proventriculus is similar in size and structure to that of *Dissosteira carolina* Linnaeus and *Dendrotettix quercus* Packard and shows the longitudinal ridges (Fig. 72—LR) and fine scalelike projections (S) of the crop and the longitudinal plates of the proventriculus (LP). It is, however, less robust than that in the aforementioned species, having very fine scales (S) in the crop and comparatively fewer scales on the longitudinal plates.

Syrbula admirabilis Uhler

Eritettix simplex tricarinatus Thomas

Amphitornus coloradus ornatus Scudder

Amblytropidia occidentalis Saussure

Orphulella pelidna Burmeister

Dichromorpha viridis Scudder

Clinocephalus elegans Morse

Chorthippus curtipennis Harris

Ageneotettix deorum Scudder

In these species the proventriculus is similar in structure to that of *Chloealtis conspersa* Harris.

In all the species of Acrididae investigated the proventriculus shows a common form throughout, any differences being in the relative robustness of the structures. In the larger forms such as *Romalea microptera* Beauvais the longitudinal plates are large and bear many scalelike projections while in smaller forms such as *Chloealtis conspersa* Harris the plates are small and less heavily sclerotized and bear fewer scalelike projections.

Terms Used by Authors in Describing the Proventriculus of Acrididae

Longitudinal ridge of crop:

—longitudinal fold (Bordas)

—longitudinal ridge (Dufour, Hodge)

Longitudinal plate of proventriculus:

—chitinous plate (Bordas)

—tooth of gizzard (Dufour)

—arrow-shaped plate (Faussek)

—proventricular tooth (Hodge, 1936)

—proventricular plate (Hodge, 1940)

TETRIGIDAE

Historical Note

Carpentier (27) described the external features of the alimentary canal of *Acrydium Kiefferi* but gave no details on the internal structure.

Original Descriptions

Batrachidinae

Tettigidea lateralis parvipennis Harris

Externally the proventriculus (Fig. 9—P) is a tubular structure continuous with the crop (C) and entering the mid-gut (MG) between the bases of the six gastric caeca (GC). The caeca project forward from the anterior end of the mid-gut and are about 1 mm. long. The lining of the proventriculus is a thin intima with a few weak longitudinal folds posteriorly.

Longitudinal section (Fig. 79).—The inner lining of the crop and proventriculus is the very thin intima (I), which consists of an inner layer 2 to 3 μ thick and an outer layer 5 to 10 μ thick. The epithelial layer (EP) is one cell thick and continuous with the epithelial layer of the gastric caeca (GC) and oesophageal valve (Oes. V.). The circular muscle (CM) is present as a few scattered fibers in the crop and is two to three fibers thick in the proventriculus and oesophageal valve.

Tetriginae

Paratettix cucullatus Burmeister

The proventriculus is similar to that of *Tettigidea lateralis*. Its lining is uniformly smooth and posteriorly shows six longitudinal folds that are weakly defined (Fig. 80—LF).

TRIDACTYLOIDEA

TRIDACTYLIDAE

Historical Note

Carpentier (27) described the external features of the digestive tract of *Tridactylus thoracicus* Guérin.

Original Description

Tridactylus apicalis Say

The proventriculus (Fig. 10—P) forms a tubular structure with the crop (C) and enters the mid-gut (MG) between two bulbous gastric caeca (GC). These gastric caeca project forward from the anterior end of the mid-gut and

are about 0.5 mm. long.   The inner lining of the proventriculus (Fig. 82—I) is thin and continuous with that of the crop (C) and shows a few weak longitudinal ridges where it constricts to form the oesophageal valve.

Transverse section (Fig. 83).—The proventriculus lies between the two gastric caeca (GC).   Its inner lining is the intima (I), 2 to 5 μ thick and raised in irregular longitudinal folds.   The epithelial layer (EP) is composed of a single layer of cells.   The circular muscle (CM) is three to four fibers thick.

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CYLINDRACHAETIDAE

Historical Note

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Carpentier (27) described the external features of the digestive tract of *Cylindroryctes spegazzini* but gave no details on the internal structure.

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Original Description

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Cylindroryctes spegazzini Giglio-Tos

The crop (Fig. 8—C) and proventriculus (P) are tubular and enter the mid-gut (MG) between the bases of the six gastric caeca (GC).   These gastric caeca project forward from the anterior end of the mid-gut and are 2 mm. long.   The lining of the proventriculus (Fig. 81—P), in common with that of the crop (C), is uniformly smooth and unrelieved by any projections. As it narrows toward the oesophageal valve (Oes. V.) the lining forms weak transverse furrows.

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G R Y L L O B L A T T A R I A

GRYLLOBLATTIDAE

Original Description

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Grylloblatta campodeiformis Walker

The proventriculus consists of a globular body (Fig. 11—P) and a tubular neck (N) joined to the crop (C).   The globular body enters the mid-gut (MG) between two short gastric caeca (GC).   The lining of the neck and anterior part of the body of the proventriculus shows 12 longitudinal primary folds (Fig. 85—PF), which bear an armature of fine scalelike points (S).   In the posterior half of the body of the proventriculus the primary folds alternate with, and at the posterior border are replaced by, 12 secondary folds (SF), which likewise bear the armature of fine points.   At the posterior end of the proventriculus there are two ranks of pyramidal teeth, which project into the lumen of the proventriculus.   They bear a coat of fine hairs.   The teeth in the primary rank (PT1) are 0.3 mm. long and are borne at the posterior ends of the 12 secondary folds.   The teeth in the secondary rank (PT2) are 0.4 mm. long and are borne at the posterior ends of the primary folds.   Posterior to the ranks of teeth is the lining of the oesophageal valve (Oes. V.).

Longitudinal section (Fig. 88) *and transverse sections* (Fig. 86—through primary folds; Fig. 87—through primary and secondary folds; Fig. 89—through

primary and secondary ranks of teeth; Fig. 90—through one primary tooth and two secondary teeth; Fig. 91—through secondary rank of teeth; Fig. 92—through single secondary tooth).—The intima (I) is 10 to 15 μ thick and bears the fine scalelike points. The epithelial layer (EP) is one to four cells thick and is continuous with that of the oesophageal valve (Oes. V.) and gastric caeca (GC). The longitudinal muscle (LM) is sparse except in the pyramidal teeth (PT) where it is several fibers thick. The circular muscle (CM) is three to four fibers thick in the neck region and 8 to 10 fibers thick in the globular part.

ISOPTERA

Historical Note

Sutherland (107) studied longitudinal sections of the alimentary canals of species in six genera of Isoptera.

Original Description

TERMITIDAE

Calotermitinae

Termopsis angusticollis Hagen (worker)

The proventriculus is conical and 1 mm. long. In its anterior end are 12 teeth (Fig. 84—CT). These are narrow and have a thin plate projecting into the lumen of the proventriculus. This plate is tallest and most heavily sclerotized at the anterior end of the tooth. At each side of each tooth there is a narrow longitudinal fold (LF). Posterior to each of six alternate teeth is a large cushion (C), rounded at its anterior end, bearing small rounded tubercles, and tapering posteriorly to the oesophageal valve (Oes. V.).

Terms Used by Authors in Describing the Proventriculus of Isoptera

Sclerotized tooth:

— narrow fold (Sutherland)

Cushion:

— lower cushionlike fold (Sutherland)

DERMAPTERA

Historical Note

Bordas (9, 16) described the proventriculus of *Forficula auricularia* Linnaeus.

Original Description

LABIDURIDAE

Anisolabis maritima Buller

The proventriculus (Fig. 97—P) is a tubular structure 1.5 mm. long with its anterior end widened slightly to join the crop (C). The tubular posterior end enters the mid-gut (MG).

On the inner surface of the proventriculus the intima has six longitudinal folds (Fig. 98—LF). At its anterior end each fold bears an oval cushion (CC) projecting into the lumen and clothed with sharp bristles. The remainder of the surface of the fold is covered with rounded scalelike projections. At the posterior end of each fold is a slight constriction followed by a conical flap of the oesophageal valve (Oes. V.). This flap bears sharp bristles similar to those on the cushion at the anterior end of the longitudinal fold.

Terms Used by Authors in Describing the Proventriculus of Dermaptera
Longitudinal fold:
    —spatulate strip (Bordas)

PLECOPTERA

Historical Note

Wu (121) described the proventriculus of *Nemoura vallicularia*.

Original Description

PERLIDAE

Acroneuria abnormis (Newman) nymph

In the nymph of this insect the proventriculus is cylindrical (Fig. 12—P) and is continuous with the crop (C). It is surrounded by seven gastric caeca (GC) of the mid-gut (MG) that project forward. Of these the two lateral ones are longest, being 5 mm. long. The other five are 3 mm. long, three being ventral and two dorsal.

The proventriculus is 0.3 mm. long. The anterior part of the intima of the organ is traversed by 14 longitudinal plates beset with small spines (Fig. 93, Fig. 94—LT). Posteriorly these anastomose to form 12 plates in the posterior part of the proventriculus (Fig. 96—LT). Six of these project farther into the lumen of the proventriculus than do the six that alternate with them. Beneath the intima is a layer of small epithelial cells (Figs. 95, 96—EP). In the cavities beneath the plates are strands of longitudinal muscle (Figs. 95, 96—LM). The outer wall of the proventriculus consists of circular muscle three to four strands thick (Figs. 95, 96—CM).

Discussion

For sound phylogenetic studies it is necessary to take into account the total structure of the organisms investigated. The earlier workers on insects in this field concerned themselves mainly with the details of the external structures such as the venation of the wings and the modifications of the mouth parts. This work was carried further, in the study of orthopteroid insects, by investigation of such structures as the terminal abdominal appendages, as noted in the "Historical Review". A pioneer in the study of internal anatomy for comparative purposes was Bordas who worked on the features of the digestive tract (14, 16) and of the nervous system (18).

Internal structures have not been used extensively in taxonomic and phylogenetic studies largely because of the difficulty of dissecting them out and preparing them for study. As a result of this neglect some insects have been assigned to taxonomic groups after their external features alone have been studied, whereas investigation of the details of their internal anatomy have indicated that they should be included in some other taxonomic group. This will be most clearly illustrated later in consideration of the phylogenetic relationships of the 'sand-cricket', *Tridactylus apicalis* Say. This is an insect of fossorial habit, which bears a superficial resemblance to the 'mole-crickets' and which has therefore been assigned to the Ensifera. Closer study of the details of both external and internal anatomy indicates that it should be assigned to the Caelifera.

As indicated in the "Historical Review" several authors have based their phylogenetic schemes upon investigations of the fossil record. Remains and impressions of internal structures are lacking in fossils, and consequently deductions concerning phylogeny based upon internal characters can be made only from the study of insects now existing. The existence, in the past, of ancestral groups of orthopteroid insects, such as "Protoblattoidea" and "Protorthoptera" is deduced mainly from the study of fossils and of the external characters of insects now existing.

For purposes of comparison reference may be made to diagrams illustrating the phylogenetic schemes of various authors: Walker (112), Crampton (35), Handlirsch (in Schröder (101), 1925), Karny (75) (in Ander (1)), Ander (1, 2), Zeuner (122).

BLATTODEA

In the cockroaches investigated in this study the proventriculus is conical, with the crop joining the cone at its broad base, and with the apex of the cone entering the mid-gut as an oesophageal valve surrounded by the eight tubular gastric caeca of the mid-gut. The structure of the intima likewise shows a common pattern. The anterior portion is occupied by six sclerotized teeth alternating with varying numbers of folds or spine-bearing patches. Posterior to each tooth there are one or two cushions followed by the oesophageal valve.

MANTODEA

In all the species of mantids investigated the proventriculus shows a common structure. It is conical in shape with the broad end of the cone leading from the crop, and the apex of the cone entering the mid-gut as the oesophageal valve. At the anterior end of the mid-gut are eight tubular gastric caeca immediately posterior to the proventriculus. The anterior portion of the inner lining of the proventriculus is occupied by six teeth running longitudinally, and by six areas of anastomosing ridges alternating with the teeth.

Posterior to each tooth there are two cushions followed by the oesophageal valve. In the species of the four subfamilies studied there are no evident differences of structure by which to separate the subfamilies one from another.

The gross structure of the proventriculus of the Mantodea is very similar to that of the Blattodea and differs only in that there are areas of anastomosing ridges between the teeth of the former rather than series of longitudinal folds as in the latter. This similarity of structure leads to the conclusion that the Mantodea and Blattodea are closely related, a conclusion amply supported by the work of various authors on other structures: Bordas (14, 16)—alimentary canal; Bordas (18)—nervous system; Walker (111, 112)—terminal abdominal appendages; Crampton (29, 35)—external characters; Ford (53)—abdominal musculature; Handlirsch (57) and Zeuner (122)—fossil record; Nesbitt (83)—nervous system. The common ancestry of the two orders is shown in their being assigned by Handlirsch (in Zeuner, 122) to a superorder Blattaeformia derived from the "Protoblattoidea".

ISOPTERA

In the form studied in this order, *Termopsis angusticollis* Hagen, the proventriculus is similar to that of the Blattodea and Mantodea. It is conical, and adjacent to its posterior end are eight tubular gastric caeca of the mid-gut. Its inner lining resembles more closely that of the Blattodea than of the Mantodea as it has longitudinal folds rather than anastomosing ridges between the teeth. It differs, however, from both orders in having 12 rather than six teeth in its anterior portion.

The work of Sutherland (107) on six other genera of termites indicates that they are closely related to the Blattodea. She studied longitudinal sections of the whole digestive tracts and her figures are similar to that of the writer's figure of *Parcoblatta pennsylvanica* DeGeer (Fig. 14), showing the conical form of the proventriculus, the teeth, and the cushions posterior to the teeth. The close relationship of the Isoptera to the Blattodea and Mantodea is agreed to by several authors: Walker (111, 112)—terminal abdominal appendages; Crampton (35)—external characters; Handlirsch (57) and Zeuner (122)—fossil record; Nesbitt (83)—nervous system; Rau (93)—habits and oviposition. The three orders are assigned to a common superorder "Blattaeformea" by Handlirsch (in Zeuner (122)).

ORTHOPTERA

ENSIFERA

GRYLLOIDEA

In the Grylloidea the globular body of the proventriculus is joined to the crop by a tubular neck and lies between the two bulbous gastric caeca. Internally

the neck bears six longitudinal rows of cushions with hairs, and the globular part has six corresponding longitudinal folds bearing a series of appendages. A well-developed appendage is composed of a median tooth with median denticles, two lateral teeth, two lateral denticles, and two inner barbated lobes. At each side of an appendage there is an outer barbated lobe and each longitudinal fold is flanked by a partition coextensive with it. A flap of the oesophageal valve is situated at the end of each longitudinal fold.

Within the Grylloidea the two families Gryllotalpidae and Gryllidae show differences in structure that are characteristic. In the Gryllotalpidae there is no median posterior projection of the median tooth and the median denticles are represented by either a single tubercle (*Gryllotalpa*) or a row of short median denticles (*Scapteriscus*). In the Gryllidae there is a posterior projection of the median tooth with several median denticles.

TETTIGONIOIDEA

In this group the proventriculus is similar to that of the Grylloidea in consisting of a globular body lying between the two bulbous gastric caeca and a tubular neck joining the body to the crop. Internally there are six longitudinal rows of cushions in the neck and six longitudinal folds, bearing appendages, in the globular part. Each appendage is flanked by a barbated lobe at each side, and each longitudinal fold by a partition. The structure of the appendages, however, differs from that in the Grylloidea in being less complicated. Each well developed appendage consists of a median tooth sometimes with lateral projections, and two lateral lobes. The lateral teeth and inner barbated lobes, present in the Grylloidea, are not present in the Tettigonioidea.

That the Tettigonioidea and the Grylloidea are closely related is a conclusion reached by many authors: Bordas (16)—digestive tract; Bordas (18)—nervous system; Walker (111, 112)—terminal abdominal appendages; Crampton (35)—external features; Handlirsch (57) and Zeuner (122)—fossil record; Ander (1)—external features; Nesbitt (83)—nervous system.

Within the superfamily Tettigonioidea the proventriculus shows considerable variety of structure. The five subfamilies of the Tettigoniidae studied may be divided into two groups on the basis of the structure of the proventriculus: I. Phaneropterinae—in this subfamily the appendages are covered with hairs and the barbated lobes have a small spine surrounded by a tuft of hairs; II. Phasgonurinae, Copiphorinae, Conocephalinae, Decticinae—the appendages have no hairs and the barbated lobes are strong sclerotized structures without hairs.

Considerable difference of opinion exists on the question of the relationships of the groups of 'gryllacrid' insects to one another. One point of view is that of Karny (75) who places all these in a single family Gryllacrididae with

subfamilies Stenopelmatinae, Henicinae, Prophalangopsinae, Deinacridinae, and Rhaphidophorinae. Ander (1), on the other hand, claims that there are several families, e.g. Prophalangopsidae, Rhaphidophoridae, and Stenopelmatidae (subfamilies Stenopelmatinae and Henicinae). The structure of the proventriculus in these insects indicates that the gryllacrids are not a homogeneous group and that Ander's classification indicates the true relationships. In the Rhaphidophoridae the appendages consist of a median tuft of hairs with lightly sclerotized lateral lobes. In the Stenopelmatidae and Prophalangopsidae the appendages are strongly sclerotized and have no median tufts of hairs. They resemble the Tettigoniidae more closely than they do the Rhapidophoridae. Ander (1, 2) sums up this relationship by including the Tettigoniidae, Stenopelmatidae, and Prophalangopsidae in a group "Tettigonaeomorpha".

CAELIFERA

In the members of the four families of this group the proventriculus is comparatively simple in structure, being tubular in shape and having no heavy armature on the intima. In the Acrididae the longitudinal folds of the crop converge posteriorly to form six longitudinal plates in the proventriculus, each bearing small scalelike projections. In the Tetrigidae the crop shows no longitudinal folds in its intima, but has six weakly defined longitudinal folds in the proventriculus. In the Tridactylidae and Cylindrachaetidae the thin intima of the crop and proventriculus shows no folds or projections.

The gastric caeca, closely associated with the proventriculus, vary in number and form in the four families. In the Acrididae there are six gastric caeca, each with a long tubular projection forward from its point of attachment to the mid-gut and a shorter tubular projection backward from its point of attachment. In the Tetrigidae and Cylindrachaetidae there are six gastric caeca projecting forward from the mid-gut and there are no posterior projections. In the Tridactylidae there are two gastric caeca projecting forward from the mid-gut and there are no posterior projections.

On the basis of external and internal characters the Acrididae and Tetrigidae are clearly seen to be closely related as members of the suborder Caelifera. In the proventriculus the simple tubular structure prevails in both and both show the six longitudinal posterior folds or plates, better developed in the Acrididae. The work of various authors on other structures confirms this view: Robertson (97)—chromosomes; Walker (111, 112)—terminal abdominal appendages; Ford (53)—abdominal musculature; Carpentier (27)—digestive tract.

The Tridactylidae and Cylindrachaetidae have long been considered as closely related to the Gryllidae and therefore have been assigned to the Ensifera (e.g. Essig (47), Schröder (101, 1929)). This was due to the fact

that these insects are burrowing forms and the legs and body are adapted to fossorial life, and they resemble externally such insects as the fossorial *Gryllo-talpa* of the Ensifera. Closer investigation of the external anatomy by Carpentier (25, 26) led to the conclusion that they were allied to the Caelifera, and the study of the external characters of the digestive tract (Carpentier (27)) led to the same conclusion.

The proventriculus is definitely of the type found in the Caelifera and has nothing in common with that of the Ensifera. In *Tridactylus* and *Cylindroryctes* the intima is thin and smooth and unrelieved by any regular folds or projections.

The Caelifera and Ensifera have been assigned to the order Saltatoria (e.g. Handlirsch (57)). Ander (2), however, claims that the Caelifera form "eine selbständige Linie" independent of the Ensifera and (1) that "die beiden Unterordnungen der Saltatoria auf verschiedene Protorthopteren—stämmen zurückgehen". The striking difference in the form of the proventriculus and in the form and number of the gastric caeca in the two suborders confirms this view.

PHASMIDA

In the phasmids the proventriculus is a tubular structure continuous with the crop and with the oesophageal valve, which projects as a long flap into the mid-gut. Its only armature on the intima consists of the series of longitudinal folds beset with small spines or scalelike projections.

A difference of opinion on the phylogenetic position of the Phasmida is evident, some authors assigning the group to the Protorthoptera and some to the Protoblattoidea. Ford (53) and Walker (112) consider that the phasmids arise from the base of the stem culminating in the Blattaria and Mantaria, and Nesbitt (83) groups them with the Blattidae and Mantidae on the basis of the number of recurrent nerves. Handlirsch (57), Crampton (35), and Zeuner (122) conclude that the phasmids arise from the Protorthoptera.

The proventriculus of the phasmids does not resemble that of the Ensifera, Acridoidea, or Blattaeformia but is considerably less complicated in structure than in these groups, and there are associated with it no well-defined gastric caeca that might give a clue to its relationship with these groups. The simplicity of its structure leads to the conclusion that the Phasmida are primitive types as compared with other orthopteroid insects but it gives no indication of the relationship of the Phasmida to other groups.

GRYLLOBLATTARIA

In *Grylloblatta campodeiformis* Walker the proventriculus consists of a globular body and anterior to this a short tubular neck. In the anterior

part of the body the sclerotized intima has 12 longitudinal folds bearing spines, and in the posterior part 12 similar folds that alternate with the posterior ends of those in the anterior part. The part of the proventriculus immediately anterior to the oesophageal valve is occupied by two ranks of pyramidal teeth with 12 teeth in each rank. The globular body of the proventriculus lies between two small gastric caeca.

Since the discovery of this insect by Walker its systematic position has been the subject of much discussion. Crampton (28) assigned the insect to an order Notoptera, and in a later paper (30) concluded that it was related to the Embiids and still later (33) considered that it was related to such forms as the Ensifera. Imms (70) grouped the insect with the "Cursoria", which included the Blattidae and Mantidae. Walker (111, 112, 113, 114, 116, 117) made detailed studies of various structures of *Grylloblatta*. He concluded (116) that it had closer affinities with the Saltatoria such as *Gryllus* than with other groups, but showed considerable resemblance to Plecoptera as represented by *Nemoura vallicularia*. He emphasized (117) the relationship of the Grylloblattaria with the Saltatoria, especially the Ensifera, rather than with the Blattodea, Mantodea, and Isoptera.

The external features of the proventriculus of *Grylloblatta* resemble most closely those of the Ensifera, there being a globular body joined to the crop by a tubular neck. There are, however, no well-developed gastric caeca such as are found in the Ensifera. The anterior end of the mid-gut is invaginated slightly to accommodate the body of the proventriculus and may show two slight projections forward laterally, comparable to the gastric caeca of the Ensifera.

While the proventriculus of *Grylloblatta* does resemble that of the Ensifera in its external appearance it in nowise resembles it in internal structure. The 12 longitudinal folds and the two ranks of pyramidal teeth in the proventriculus of *Grylloblatta* are quite unique. The group that it most resembles is the Plecoptera in that it has the longitudinal folds bearing small spines. But it does not have the tubular shape of the proventriculus of the Plecoptera and there are not the seven gastric caeca as in the mid-gut of the Plecoptera.

The structure of the proventriculus of *Grylloblatta* then, does not indicate close relationship to any of the orthopteroid insects but it suggests that the Grylloblattaria arose from the stem giving rise to the Ensifera.

DERMAPTERA

In *Anisolabis maritima* Buller, the proventriculus is a tubular structure with its anterior end flared slightly where it joins the crop. On its inner surface there are six longitudinal folds bearing small scalelike projections with a cushion of bristles at the anterior end of each fold.

The proventriculus in this species does not resemble closely that of any other of the orthopteroid insects studied. However, it is somewhat similar to the proventriculus of the Blattodea. The six cushions bearing bristles are comparable to the ovoid patches of *Pycnoscelus surinamensis* Linnaeus and the longitudinal folds are somewhat comparable to the longitudinal folds leading to the oesophageal valve in several species of Blattodea. But the proventriculus does not have the conical structure of the Blattodea, nor does the mid-gut have the eight tubular gastric caeca characteristic of the Blattodea, Mantodea, and Isoptera.

The phylogenetic position of the Dermaptera has been discussed by several authors. Walker (112) derives the group from the Paleodictyoptera independently of the other orthopteroid groups, but closest to the Plecoptera. Ford (23) says "the arrangement of the muscles also confirms the relationship of the Dermaptera with the Plecoptera". Crampton (35) in his "phylogenetic tree" groups the Dermaptera with the Orthoptera and Phasmids but admits that they have some characters in common with the blattoid insects. Nesbitt (83) groups the Dermaptera with the blattoid insects on the basis of the possession of one posterior recurrent nerve. The structure of the proventriculus does not throw any deciding light on the subject but does indicate that the Dermaptera may be related to the Blattoid insects.

PLECOPTERA

In the form studied in this group, *Acroneuria abnormis*, the proventriculus is tubular and bears on its inner surface 14 longitudinal plates that anastomose posteriorly to form 12 longitudinal ridges. There are seven tubular gastric caeca projecting forward from the mid-gut and surrounding the proventriculus.

The phylogeny of the Plecoptera has been discussed by several authors. Walker (112) derives this group from the Palaeodictyoptera independently of the other orthopteroid insects. Ford (53) says that "the arrangement of the muscles also confirms the relationship of the Dermaptera with the Plecoptera . . . ." Crampton (35) considers the Plecoptera to be a group derived from the Palaeodictyoptera independently of the other orthopteroid insects.

The structure of the proventriculus is unlike that of any other of the orthopteroid insects studied except that its inner plates resemble those of *Grylloblatta*, and the presence of seven gastric caeca is unique. On the basis of the structure of this organ, then, it is to be concluded that the Plecoptera form a group independent of the other orthopteroid insects.

Phylogeny

The relationships between the groups of orthopteroid insects arrived at in this study are expressed in the following table:

Dermaptera

Isoptera

Protoblattoidea ——Blattodea

Mantodea

P
A——————————————Phasmida　　　　Tridactylidae ⎱
L　　　　　　　　　　　　　　　　　　　　　　　　　　⎰ Tridactyloidea
A　　　　　　　　　　　　　　　　　　Cylindrachaetidae ⎰
E——————————————Caelifera
O
D　　　　　　　　　　　　　　　　　　Acrididae ⎱
I　　　　　　　　　　　　　　　　　　　　　　　　 ⎰ Acridoidea
C　　　　　　　　　　　　　　　　　　Tetrigidae ⎰
T
Y　　　　　　　　　　　　　　　　　　Gryllidae ⎱
O　　　　　　　　　　　　　　　　　　　　　　　　 ⎰ Grylloidea
P　　　　　　　　　　　　　　　　　　Gryllotalpidae ⎰
T
E——————————————Ensifera —————— Rhaphidophoridae ⎱
R　　　　　　　　　　　　　　　　　——— Stenopelmatidae ⎱
A　　　　　　　　　　　　　　　　　　　　　　　　　　　　　 Tettigonioidea
　　　　　　　　　　　　　　　　　– Prophalangopsidae ⎰
　　　　　　　　　　　　　　　　　Tettigoniidae ⎰

Grylloblattaria
Plecoptera
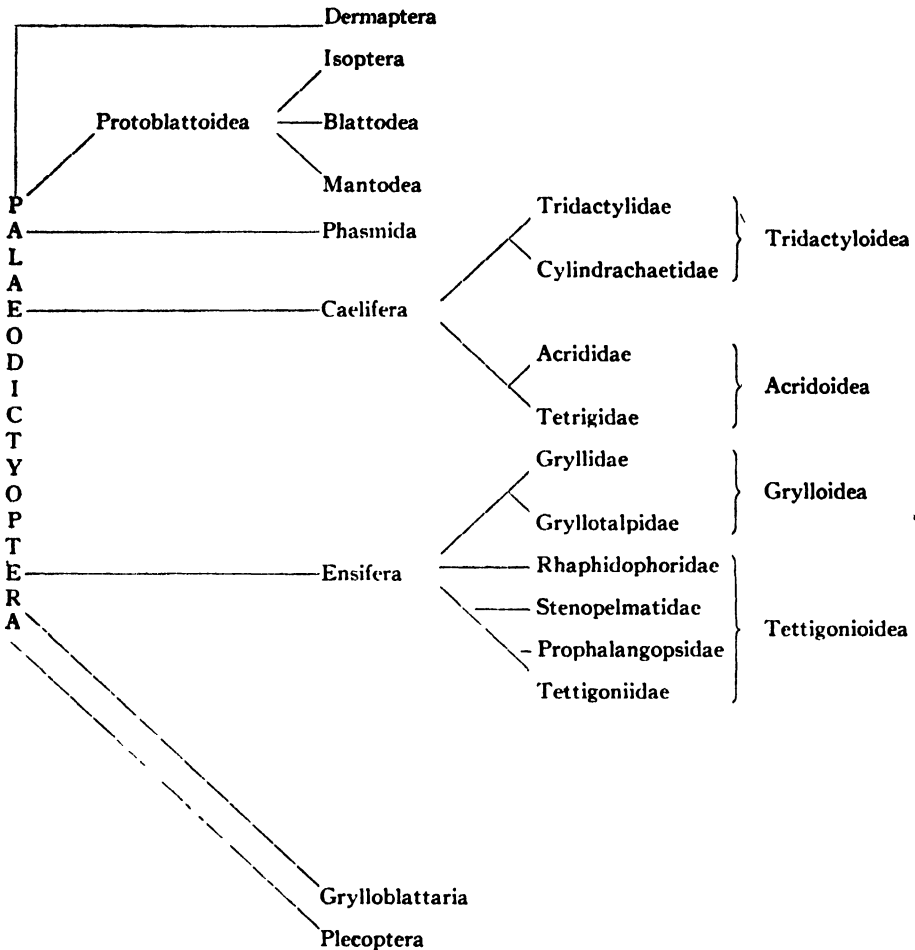
The "Palaeodictyoptera" is the ancestral form, proposed by Handlirsch (56), from which all winged insects are derived. The main argument for the existence of such a common ancestor for insects is the presence of the two pairs of wings on the same two body segments (mesothorax and metathorax) of all winged insects and the common pattern of the principal veins of the wings.

Taxonomy

The diversity of structure displayed by the proventriculus of insects has led several authors to base keys for identification of genera on the characters of this organ. Emery (46) composed a "Stammbaum" to show the relationships between genera of the families Dolichoderidae and Camponotidae of ants. Ris (94), basing his results on the structure of the proventriculus of nymphal and adult dragonflies, composed a table to show the relationship of eight families of Odonata to one another. Higgins (64) says of Ris's work

that "classification into genera based on resemblances ,in gizzard structure would agree in most cases with that now in use based on structure of wings and other external features of the body".

The work of Felt (51) and Swaine (108) on Scolytidae has led these authors to conclude that the proventriculus of these insects is of value in taxonomy; and Swaine says, "I have found . . . . the proventriculus of the greatest interest and much practical value; but a wider study is apparently necessary . . . . before definite conclusions can be drawn". For the Dytiscidae Balfour-Browne (3) arranged a key, based on the structure of the proventriculus, for the separation of 22 genera; and in another paper (4) he showed that the divisions of Dytiscidae based on the structure of this organ coincided with divisions based on the structure of the tarsal claws. Sutherland (107) concluded that "the alimentary canals of termites of different families can be arranged in a definite series, corresponding to their systematic positions".

The following key, for the forms included in this study, is based upon the external and internal features of the proventriculus and the gastric caeca.

A. Proventriculus conical, intima with 6 or 12 teeth; eight tubular gastric caeca
    B. Sclerotized folds between teeth . . . . .Blattodea, Isoptera
    BB. Anastomosing ridges between teeth . . . . .Mantodea
AA. Proventriculus globular, with tubular neck
    B. Twelve longitudinal folds on intima, two ranks of 12 pyramidal teeth at posterior end; no prominent gastric caeca . . . . .Grylloblattaria
    BB. Six longitudinal folds on intima with series of sclerotized appendages; longitudinal folds separated by partitions; two bulbous gastric caeca lying laterad to proventriculus
        C. Appendages with median tooth, median denticles, lateral teeth, lateral denticles, inner and outer barbated lobes .Grylloidea
            D. No posterior projection of median tooth; single median denticle or row of denticles. . . . . .Gryllotalpidae
            DD. Posterior projection of median tooth with several median denticles . . . . .Gryllidae
        CC. Appendages with median tooth, lateral lobe, and single barbated lobe . . . . .Tettigonioidea
            D. Median tooth bearing a tuft of hairs; lateral lobes lightly sclerotized . . . . .Rhaphidophoridae
            DD. Median tooth without tuft of hairs; tooth heavily sclerotized . . . . .Tettigoniidae, Stenopelmatidae, Prophalangopsidae
AAA. Proventriculus tubular
    B. No gastric caeca
        C. Six longitudinal folds on intima, each with an anterior cushion of bristles . . . . .Dermaptera
        CC. Numerous longitudinal folds on intima . . . . .Phasmida
    BB. Two or six gastric caeca
        C. Six longitudinal plates in the proventriculus; six gastric caeca, each with anterior and posterior projections. . . . . .Acrididae
        CC. No longitudinal plates in proventriculus; gastric caeca with anterior projection only
            D. Two gastric caeca . . . . .Tridactylidae
            DD. Six tubular gastric caeca .Cylindrachaetidae
            DDD. Six conical gastric caeca . . . . .Tetrigidae
    BBB. Seven gastric caeca; 14 longitudinal plates in the proventriculus . . . . . .Plecoptera

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References

1. ANDER, K. Vergleichend—anatomische und phylogenetische Studien über die Ensifera. Opuscula Entomologica, Supplementum 2. Berlingska Boktryckeriet, Lund. 1939.
2. ANDER, K. Systematische Einteilung und Phylogenie der Ensifera auf Grund von vergleichend anatomischen Untersuchungen. Verhandl. 7 intern. Kongr. Entomol., Berlin, 2 : 621-627. 1939.
3. BALFOUR-BROWNE, F. The proventriculus in the Dytiscidae as a taxonomic character. Stylops, London, 3 : 241-244. 1934.
4. BALFOUR-BROWNE, F. The proventriculus in the Dytiscidae as a taxonomic character, second note. Stylops, London, 4 : 191. 1935.
5. BASCH, S. Untersuchungen über das chylopoetische und uropoetische System der *Blatta orientalis*. Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, 33 : 234-260. 1858.
6. BERLESE, A. Osservazioni sulla anatomia descrittiva del *Gryllus campestris*. Atti accad. sci. Veneto-Trentino-Istriana, 7 : 200-299. 1880.
7. BERLESE, A. Gli Insetti, Vol. 1. Milano Societa Editrice Libraria. 1907.
8. BLOESER, W. Notes on the life history and anatomy of *Siphona plusiae* Coq. (Tachinidae). Ann. Entomol. Soc. Am. 7 : 301-309. 1914.
9. BORDAS, L. Anatomie de l'appareil digestif des Orthoptères de la famille des *Forficulidae*. Compt. rend. 121 : 655-657. 1895.
10. BORDAS, L. Appareil digestif des Blattidae (*Periplaneta americana* et *Periplaneta orientalis*). Bull. muséum natl. hist. nat. (Paris), 2 : 271-274. 1896.
11. BORDAS, L. Appareil digestif d'un Orthoptère de la famille des Gryllidae, le *Brachytrypes membranaceus*. Compt. rend. 122 : 1553-1556. 1896.
12. BORDAS, L. Considérations générales sur l'appareil digestif des Phasmidea. Bull. muséum natl. hist. nat. (Paris), 2 : 378-379. 1896.
13. BORDAS, L. Etude de l'armature masticatrice du gésier chez les Blattidae et les Gryllidae. Compt. rend. 123 : 271-273. 1896.
14. BORDAS, L. Classification des Orthoptères d'après les caractères tirés de l'appareil digestif. Compt. rend. 124 : 821-823. 1897.
15. BORDAS, L. Anatomie de l'appareil digestif des Acridiens formant la tribu des Pamphaginae, *Pamphagus elephas* Stal. Zool. Anz. Leipzig, 20 : 57-59. 1897.
16. BORDAS, L. L'appareil digestif des orthoptères et son importance pour la classification des Orthoptères. Ann. sci. nat. zool. 5 : 1-192. 1898.
17. BORDAS, L. Etude sur l'appareil digestif du *Brachytrupes achatinus* Stoll. Compt. rend. 131 : 66-69. 1900.
18. BORDAS, L. Contribution à l'étude du système nerveux sympathique sus-intestinal ou stomatogastrique des Orthoptères. Bull. biol. France Belg. 33 : 458-482. 1900.
19. BOYCOTT, A. E. Physiological anatomy of the mouth-parts and alimentary canal of the Indian rat flea—*Pulex cheopis* Rothschild. J. Hyg. 6 : 486-495. 1906.

20. BREAKEY, E. P. Histological studies of the digestive system of the squash bug, *Anasa tristis* de G. (Hemiptera, Coreidae). Ann. Entomol. Soc. Am. 29 : 561-577. 1936.

21. BRUNETTI, B. Sulla funzione del proventricolo degli insetti. Atti soc. tosc. sci. nat. Pisa, 38 : 24-27. 1929.

22. BRUNETTI, B. Ricerche sul proventricolo degli insetti. Morfologia del proventricolo. Formazioni chitinose. Rapporto fra la struttura delle formazioni chitinose ed il regime alimentare. Atti soc. tosc. sci. nat. Pisa, 41 : 110-146. 1931.

23. BURMEISTER, H. Handbuch der Entomologie. Vol. 2. Berlin. 1838.

24. CAMERON, A. E. Structure of the alimentary canal of the stick-insect, *Bacillus rossii* Fabr. Proc. Zool. soc. London : 172-182. 1912.

25. CARPENTIER, F. Sur quelques particularités du thorax et des pattes de Cylindroryctes spegazzini Giglio-Tos. Fifth Congr. Intern. Entomol. Paris, 1932. II. Travaux : 271-276. 1933.

26. CARPENTIER, F. Le thorax et ses appendices chez les vrais et les faux Gryllotalpides. Mém. muséum hist. nat. Belg. (sér. 2), 4 : 1-86. 1936.

27. CARPENTIER, F. Sur le tube digestif des Tridactyloides. Bull. Ann. Soc. Entomol. Belg. 77 : 283-291. 1937.

28. CRAMPTON, G. C. The thoracic sclerites and the systematic position of *Grylloblatta campodeiformis* Walker, a remarkable annectant "orthopteroid" insect. Entomol. News, 26 : 337-350. 1915.

29. CRAMPTON, G. C. The lines of descent of the lower pterygotan insects, with notes on the relationships of the other forms. Entomol. News, 27 : 244-258, 297-307. 1916.

30. CRAMPTON, G. C. A comparison of the antennae of the Grylloblattidae and Embiidae to demonstrate the relationships of these two groups of insects. Can. Entomol. 49 : 213-217. 1917.

31. CRAMPTON, G. C. Notes on phylogeny of the Orthoptera. Entomol. News, 30 : 42-48. 1919.

32. CRAMPTON, G. C. The affinities of Grylloblatta indicated by a study of the head and its appendages. Psyche, Boston, 33 : 78-85. 1926.

33. CRAMPTON, G. C. The abdominal structures of the orthopteroid family Grylloblattidae and the relationship of the group. Pan-Pacific Entomol. 3 : 115-135. 1927.

34. CRAMPTON, G. C. Anatomical evidence that Cylindrachaeta is a gryllotalpoid, not an embiid. Entomol. Mitt. Berlin, 17 : 252-257. 1928.

35. CRAMPTON, G. C. The interrelationships and lines of descent of living insects. Psyche, Boston, 45 : 165-181. 1938.

36. CUÉNOT, L. Etudes physiologiques sur les Orthoptères. Arch. biol. 14 : 293-341. 1896.

37. DAVIS, A. C. Studies of the anatomy and histology of *Stenopelmatus fuscus* Hald. Univ. Calif. Pub. Entomol. 4 : 159-208. 1927.

38. DEAN, R. W. The alimentary canal of the apple maggot, *Rhagoletis pomonella* (Walsh) (Dipt.: Trypetidae). Ann. Entomol. Soc. Am. 25 : 210-223. 1932.

39. DEGEER, C. Mémoires pour servir à l'histoire des insectes, Vol. 3, Stockholm. 1773.

40. DESINÉTY, L. Recherches sur la biologie et l'anatomie des Phasmes. Cellule, Louvain, 19 : 118-278. 1901.

41. DOMINIQUE, J. Le tube digestif des Orthoptères. Bull. soc. sci. nat. Ouest, Nantes, 4 : 17-26. 1894.

42. DUFOUR, L. Recherches anatomiques et physiologiques sur les Orthoptères, les Hyménoptères et les Névroptères. Mém. acad. sci. Paris, 7 : 265-647. 1841.

43. DUPORTE, E. M. On the structure and function of the proventriculus of *Gryllus pennsylvanicus* Burm. Psyche, Boston, 25 : 117-122. 1918.

44. EBERLI, J. Untersuchungen am Verdauungstraktus von Gryllotalpa vulgaris. Vierteljahresschr. naturforsch. Ges. Zürich, 37 : 167-212. *Reviewed in* J. Roy. Microscop. Soc. 1892 : 781. 1892.

45. EIDMANN, H. Untersuchungen über die Morphologie und Physiologie des Kaumagens von *Periplaneta orientalis* L. Z. wiss. Zool. A, 122 : 281-309. 1924.

46. EMERY, C. Über den sogennenten Kaumagen einiger Ameisen. Z. wiss. Zool. A, 46 : 378-412. 1888.

47. ESSIG, E. O. College entomology. The Macmillan Co., New York. 1942.

48. FAASCH, W. J. Darmkanal und Blutverdauung bei Aphanipteren. Z. wiss. Biol. Abt. A. Z. Morphol. Ökol. Tiere, 29 : 559-584. 1935.

49. FABRICIUS, J. C. Systema Entomologiae sistens Insectorum classes, ordines, genera, species, adjectis synonomis, locis, descriptionibus observationibus. Flensburgi et Lipsiae. 1775.

50. FAUSSEK, V. Beiträge zur Histologie des Darmkanals der Insekten. Z. wiss. Zool. A, 45 : 694-712. 1887.

51. FELT, E. P. Insects affecting park and woodland trees. N.Y. State Museum Mem. 8, 2 : 333-877. 1906.

52. FISHER, R. C. The genus Scolytus in Great Britain, with notes on the structure of *Scolytus destructor* Ol. Ann. Applied Biol. 24 : 110-130. 1937.

53. FORD, N. A comparative study of the abdominal musculature of orthopteroid insects. Trans. Roy. Can. Inst. 14 : 207-319. 1923.

54. GRABER, V. Zur näheren Kenntniss des Proventriculus und der Appendices ventriculares bei den Grillen und Laubheuschrecken. Sitzber. Akad. wiss. Wien, Math.-naturw. Klasse, 59 : 29-46. 1869.

55. HANDLIRSCH, A. Zur Phylogenie der Hexapoden. (Vorläufige Mitteilung). Sitzber. Akad. wiss. Wien, Math.-naturw. Klasse, Abt. 1, 112 : 716-738. 1903.

56. HANDLIRSCH, A. Die Fossilen Insekten und die Phylogenie der rezenten Formen. Leipzig. 1908.

57. HANDLIRSCH, A. Phylogenie oder Stammesgeschichte; in C. Schröder's Handbuch der Entomologie. Gustav Fischer, Jena. 1925.

58. HARRIS, C. S. The anatomy and histology of the alimentary system of the Harlequin cabbage bug, *Murgantia histrionica* Hahn (Hemiptera, Pentatomidae). Ohio J. Sci. 38 : 316-331. 1938.

59. HASEMAN, L. The structure and metamorphosis of the alimentary canal of the larva of *Psychoda alternata* Say. Ann. Entomol. Soc. Am. 3 : 277-313. 1910.

60. HASEMAN, L. The hessian fly larva and its method of taking food. J. Econ. Entomol. 23 : 316-321. 1930.

61. HEYMONS, R. Über die Organisation und Entwicklung von *Bacillus rossii* Fabr. Sitzber. kgl. preuss. Akad. Wiss. :363-373. 1897.

62. HICKERNELL, L. M. The digestive system of the periodical cicada, *Tibicen septendecim* Linn. Ann. Entomol. Soc. Am. 13 : 223-248. 1920.

63. HICKERNELL, L. M. The digestive system of the periodical cicada, *Tibicen septendecim* Linn. III. Morphology of the system in the nymph. Biol. Bull. Marine Biol. Lab. 45 : 213-221. 1923.

64 HIGGINS, H. T. The development and comparative structure of the gizzard in the Odonata Zygoptera. Proc. Acad. Natural Sci. Phila. 53 : 126-141. 1901.

65. HOBSON, R. P. Studies on the nutrition of blow-fly larvae. 1. Structure and function of the alimentary tract. J. Exptl. Biol. 8 : 109-123. 1931.

66. HODGE, C. The anatomy and histology of the alimentary tract of the grasshopper, *Melanoplus differentialis* Thomas. J. Morphol. 59 : 423-439. 1936.

67. HODGE, C. The anatomy and histology of the alimentary tract of *Locusta migratoria* L. J. Morphol. 64 : 375-399. 1939.

68. HODGE, C. The anatomy and histology of the alimentary tract of *Radenotatum carinatum* var. *peninsulare* Rehn and Hebard. J. Morphol. 66 : 581-603. 1940.

69. HSU, Y. C. Morphology, anatomy and ethology of *Gryllus mitratus* Burm. Lingnan Sci. J. 10 : 187-216. 1931.

70. IMMS, A. D. On the affinities of the Grylloblattidae. Psyche, Boston, 34 : 36-39. 1927.

71. ISELY, F. B. Correlation between mandibular morphology and food specificity in grasshoppers. Proc. Texas Acad. Sci. 25 : 53-54. 1942.

72. JAHN, L. A. Internal anatomy of the mydas-fly. Ohio J. Sci. 30 : 85-97. 1930.

73. JENSEN, J. P. Structure and systematic importance of the spermatophores of crickets. Ann. Entomol. Soc. Am. 4 : 63-67. 1911.

74. JOHNSTON, C. E. Internal anatomy of *Icerya purchasi* (Coccidae) female. Ann. Entomol. Soc. Am. 5 : 383-388. 1912.

75. KARNY, H. H. Über das Flugelgeäder der Gryllacriden. Ein Beitrag zum Rosaschen Gesetz. Arch. zool. Italiano, 15 : 193-244. 1931.

76. KARNY, H. H. Family Gryllacridae. *In* P. Wytsman's Genera Insectorum, 206 : 1-316. 1937.

77. KARSCH, F. Die Insecten der Berglandschaft Adeli. Berlin. 1893

78. KARSCH, F.   Die Namen der ältesten Dermapteren—(Orthopteren-) Gattungen und ihre Verwendung für Familien- und Unterfamilien- Benennungen auf Grund der jetzigen Nomenclaturregeln.   Zool. Anz. Leipzig, 25 : 530-543.   1902.

79. LEACH, W. E.   Entomology.   In D. Brewster, Edinburgh Encyclopaedia, 9 : 57-172. 1815.

80. LINNAEUS, C.   Systema Naturae per regna tria naturae secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis.   10th ed. Vol. 1, Holmiae.   1758.

81. MIALL, L. C. and DENNY, A.   The structure and life history of the cockroach (Periplaneta orientalis).   London.   1886.

82. MULDER, C.   Ontleedkundige Aanteekening over Macrolyristes imperator Voll., vergleeken met eenige andere Regtvleugeliegen.   Tijdschr. Entomol. Amsterdam, 8 : 110-121.   1865.

83. NESBITT, H. H. J.   A comparative morphological study of the nervous system of the Orthoptera and related orders.   Ann. Entomol. Soc. Am. 34 : 51-81.   1941.

84. OHMACHI, F.   Preliminary note on a new system in the classification of Gryllodea. Proc. Imp. Acad. (Tokyo), 3 : 457-459.   1927.

85. OLIVIER, A. G.   Histoire naturelle, insectes.   Encyclopédie Méthodique, Paris, 8 : 550-554.   1811.

86. PATTON, W. S. and EVANS, A. M.   Insects, ticks, mites and venomous animals of medical and veterinary importance.   Part I.   H. R. Grubb Ltd., Croydon.   1929.

87. PETRUNKEWITSCH, A.   Zur Physiologie der Verdauung bei Periplaneta orientalis und Blatta germanica.   Zool. Anz.   Leipzig, 22 : 137-140.   1899.

88. PETRUNKEWITSCH, A.   Die Verdauungsorgane von Periplaneta orientalis und Blatta germanica.   Zool. Jahrb. Jena (Anat.), 13 : 171-190.   1900.

89. PLATEAU, F.   Recherches sur les phénomènes de la digestion chez les insectes.   Mém. Acad. Roy. Belg. Classe Sci. 41 : 1-124.   1874.

90. PLATEAU, F.   Notes sur les phénomènes de la digestion chez la blatte americaine. (Periplaneta americana L.).   Bull. Acad. Belg. Classe Sci. (sér. 2), 41 : 1206-1233. 1876.

91. RAMME, W.   Die Bedeutung des Proventriculus bei Coleopteren und Orthopteren. Zool. Anz. Leipzig, 38 : 333-336.   1911.

92. RAMME, W.   Die Bedeutung des Proventriculus bei Coleopteren und Orthopteren. Zool. Jahrb. Jena (Anat.). 35 : 419-456.   1913.

93. RAU, P.   Cockroaches: the fore-runners of termites (Orthoptera: Blattidae: Isoptera). Entomol. News, 52 : 256-259.   1941.

94. RIS, F.   Untersuchung über die Gestalt des Kaumagens bie den Libellen und ihren Larven.   Zool. Jahrb. Jena (Syst.) 9 : 596-624.   1897.

95. ROBERTS, H. R.   A comparative study of the sub-families of the Acrididae (Orthoptera) primarily on the basis of their phallic structures.   Proc. Acad. Natural Sci. Phila. 93 : 201-246.   1941.

96. ROBERTSON, C. W.   The metamorphosis of Drosophila melanogaster, including an accurately timed account of the principal morphological changes.   J. Morphol. 59 : 351-400.   1936.

97. ROBERTSON, W. R. B.   Chromosome studies.   II. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae.   J. Morphol. 27 : 179-331.   1916.

98. ROSS, H. H.   Notes on the digestive and reproductive systems of the German cockroach, Blatella germanica L.   Trans. Illinois State Acad. Sci. 22 : 206-216.   1930.

99. SAYCE, O. A.   On the structure of the alimentary system of Gryllotalpa australis (Erichs.), with some physiological notes.   Proc. Roy. Soc. Victoria, 11 : 113-129. 1899.

100. SCHIMMER, F.   Beitrag zu einer Monographie der Grylloidiengattung Myremcophila Latr. Z. wiss. Zool. A, 93 : 409-534.   1909.

101. SCHRÖDER, C.   Handbuch der Entomologie, Vol. 1—1928, Vol. 2—1929, Vol. 3—1925. Gustav Fischer, Jena.   1925, 1928, 1929.

102. SLIFER, E. H.   The internal genitalia of female Thrinchinae, Batrachotetriginae, Pamphaginae and Pyrgomorphinae (Orthoptera, Acrididae).   J. Morphol. 66 : 175-195. 1940.

103. SLIFER, E. H.   The internal genitalia of female Ommexechinae and Cyrtacanthacridinae (Orthoptera, Acrididae).   J. Morphol. 67 : 199-239.   1940.

104. SNODGRASS, R. E.   Notes on the internal anatomy of Peranabrus scabricollis (Thomas). J. N.Y. Entomol. Soc. 11 : 183-188.   1903.

105. SNODGRASS, R. E. Principles of insect morphology. McGraw-Hill Book Company, Inc., New York. 1935.

106. STOLL, C. Représentation des spectres ou phasmes, des mantes, des sauterelles, des grillons, des criquets et des blattes des quatre parties du monde. Amsterdam. 1787.

107. SUTHERLAND, J. L. Notes on the histology of the alimentary canal in some Australian termites. Proc. Roy. Soc. Victoria, 47 : 1-13. 1934.

108. SWAINE, J. M. Canadian bark beetles—Part II. Preliminary classification, with an account of habits and means of control. Can. Dept. Agr. Entomol. Bull. 14 : 7-143. 1918.

109. TIETZ, H. Anatomy of the digestive system of the Carolina Locust, *Dissosteira carolina.* Ann. Entomol. Soc. Am. 16 : 256-257. 1923.

110. VISART, O. Contribuzione allo studio del tubo digerente degli Artropodi. Atti. Soc. tosc. sci. nat. Pisa, 13 : 21-51. 1894.

111. WALKER, E. M. The terminal abdominal structures of orthopteroid insects: A phylogenetic study—Part I. Ann. Entomol. Soc. Am. 12 : 267-326. 1919.

112. WALKER, E. M. The terminal abdominal structures of orthopteroid insects: A phylogenetic study—Part II. Ann. Entomol. Soc. Am. 15 : 1-88. 1922.

113. WALKER, E. M. On the anatomy of *Grylloblatta campodeiformis* Walker. 1. Exoskeleton and musculature of the head. Ann. Entomol. Soc. Am. 24 : 519-536. 1931.

114. WALKER, E. M. On the anatomy of *Grylloblatta campodeiformis* Walker. 2. Comparison of head with those of other orthopteroid insects. Ann. Entomol. Soc. Am. 26 : 309-344. 1933.

115. WALKER, E. M. *Grylloblatta,* a living fossil. Trans. Roy. Soc. Can. V, 31(5) : 1-10. 1937.

116. WALKER, E. M. On the anatomy of *Grylloblatta campodeiformis* Walker. Ann. Entomol. Soc. Am. 31 : 588-640. 1938.

117. WALKER, E. M. On the anatomy of *Grylloblatta campodeiformis* Walker. 4. Exoskeleton and musculature of the abdomen. Ann. Entomol. Soc. Am. 36 : 681-706. 1943.

118. WIGGLESWORTH, V. B. Insect physiology. Methuen & Co., Ltd., London. 1934.

119. WILDE, K. F. Untersuchungen über den Kaumagen der Orthopteren. Arch. Naturgesch. Berlin, 43 : 135-172. 1877.

120. WILLIAMS, F. X. The anatomy of the larva of *Cecidomyia resinicoloides.* Ann. Entomol. Soc. Am. 3 : 45-57. 1910.

121. WU, C. F. Morphology, anatomy and ethology of *Nemoura vallicularia.* Bull. Lloyd Library Botany, Pharmacy, Materia Med. (Entomol. ser.) 3. 1923.

122. ZEUNER, F. E. Fossil Orthoptera Ensifera. Brit. Museum (Nat. History), Dept. Geol., London. 1939.

NOTE: *Figs. 1–98 will be found on pp. 143-161.*

EXPLANATION OF FIGURES

FIG. 1. Parcoblatta pennsylvanica *DeGeer—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 2. Mantis religiosa *Linnaeus—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 3. Diapheromera femorata *Say—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 4. Oecanthus nigricornis *Walker—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 5. Neoconocephalus ensiger *Harris—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 6. Ceuthophilus maculatus *Harris—proventriculus, posterior end of crop, and anterior end of mid-gut.*

C—crop; GC—gastric caecum; MG—mid-gut; N—neck; P—proventriculus.

FIG. 7. Dissosteira carolina *Linnaeus—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 8. Cylindroryctes spegazzinni *Giglio-Tos—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 9. Tettigidea lateralis parvipennis *Harris—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 10.   Tridactylus apicalis *Say—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 11.   Grylloblatta campodeiformis *Walker —proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 12.   Acroneuria abnormis *Newman— proventriculus, posterior end of crop, and anterior end of mid-gut (dorsal view).*

C—crop; GC—gastric caecum; MG—mid-gut; N—neck; P—proventriculus.

FIG. 13.   Parcoblatta pennsylvanica *DeGeer—sclerotized intima of proventriculus.*

FIG. 14.   Parcoblatta pennsylvanica *DeGeer—longitudinal section of proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 15.   Parcoblatta pennsylvanica *DeGeer—transverse section of proventriculûs through anterior cushions.*

A C—anterior cushion; CM—circular muscle; CT—sclerotized tooth; EP—epithelium; H—hairs; I—intima; LM—longitudinal muscle; MG—mid-gut; Oes. V. - oesophageal valve; PC—posterior cushion; PF—primary fold; RM—retractor muscle; S—large spine of sclerotized tooth; SF— secondary fold; Ss—small spine of sclerotized tooth.

FIG. 16.   Pycnoscelus surinamensis *Linnaeus—sclerotized intima of proventriculus.*

FIG. 17.   Blaberus atropos *Stoll— sclerotized intima of proventriculus.*

A C—anterior cushion; C—cushion; CT—sclerotized tooth; Oes. V.—oesophageal valve; OP— ovoid patch; P— patch covered with bristles; PC—posterior cushion; PF—primary fold.

FIG. 18.   Periplaneta americana *Linnaeus—sclerotized intima of proventriculus: teeth (CT) numbered from left to right, 1, 2, 3, 4, 5, 6.*

FIG. 19.   Cryptocercus punctulatus *Scudder—sclerotized intima of proventriculus.*

A C—anterior cushion; A S—anterior spine of anterior cushion; CT—sclerotized tooth; Oes. V.— oesophageal valve; PC –posterior cushion; PF—primary fold; PS posterior spine of anterior cushion; SC—secondary cushion; SF—secondary fold.

FIG. 20.   Mantis religiosa *Linnaeus—sclerotized intima of proventriculus showing form of the six teeth.*

FIG. 21.   Mantis religiosa *Linnaeus—transverse section through teeth.*

FIG. 22.   Mantis religiosa *Linnaeus—transverse section through anastomosing ridges.*

FIG. 23.   Mantis religiosa *Linnaeus- -transverse section through anterior cushions.*

A C—anterior cushion; A R—anastomosing ridges; CC—cushion of hairs; CH —hairs; CM— circular muscle; CT—sclerotized tooth; EP—epithelium; FT -forked tip; I—intima; LM—longitudinal muscle; Oes. V.—oesophageal valve; PC—posterior cushion; R—ridge.

FIG. 24.   Diapheromera femorata *Say- longitudinal section through proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 25.   Diapheromera femorata *Say—sclerotized intima of crop and proventriculus.*

FIG. 26.   Diapheromera femorata *Say—longitudinal folds of crop.*

FIG. 27.   Anisomorpha buprestoides *Stoll—longitudinal folds of crop.*

FIG. 28.   Hadenoecus puteanus *Scudder—sclerotized intima of one longitudinal fold of proventriculus.*

BL—barbated lobe; C—crop; CC—cushion of hairs; CM—circular muscle; CP—sclerotized partition; CT—sclerotized tooth; EP—epithelium; FL—flap of oesophageal valve; I—intima; Inv.—invagination into proventriculus; LF—longitudinal fold; LL—lateral lobe; LM—longitudinal muscle; MG—mid-gut; MT—median tooth; Oes. V.—oesophageal valve; P—proventriculus; S—spine.

FIG. 29.   Oecanthus nigricornis *Walker—transverse section through median tooth of sclerotized appendage.*

FIG. 30.   Oecanthus nigricornis *Walker—transverse section through median denticles of sclerotized appendage.*

FIG. 31.   Oecanthus nigricornis *Walker—transverse section through lateral teeth of sclerotized appendage.*

FIG. 32.   Oecanthus nigricornis *Walker—two longitudinal folds of intima of neck and body of proventriculus.*

FIG. 33.   Falcicula hebardi *Rehn—sclerotized intima of proventriculus.*

FIG. 34.   Phyllopalpus pulchellus *Uhler—sclerotized appendage of proventriculus.*
FIG. 35.   Cyrtoxipha columbiana *Caudell—sclerotized appendage of proventriculus.*
  CA—sclerotized appendage; CL—sclerotized lobe; CM—circular muscle; CP—sclerotized partition; EP—epithelium; I—intima; IBL—inner barbated lobe; LD—lateral denticle; LM—longitudinal muscle; LT—lateral tooth; MD—median denticle; MT—median tooth; OBL—outer barbated lobe; Oes. V.—oesophageal valve.

FIG. 36.   Gryllulus assimilis *Fabricius—longitudinal section of proventriculus.*
FIG. 37.   Scapteriscus vicinus *Scudder—sclerotized appendage of proventriculus.*
FIG. 38.   Nemobius fasciatus *DeGeer—sclerotized appendage of proventriculus.*
FIG. 39.   Gryllulus domesticus *Linnaeus—sclerotized appendage of proventriculus.*
FIG. 40.   Oecanthus nigricornis *Walker—sclerotized appendage of proventriculus.*
FIG. 41.   Scapteriscus vicinus *Scudder—sclerotized intima of one longitudinal fold.*
FIG. 42.   Hapithus brevipennis *Saussure—sclerotized appendage of proventriculus.*
FIG. 43.   Tafalisca lurida *Walker—sclerotized appendage of proventriculus.*
  CC—cushion of hairs; CM—circular muscle; CP—sclerotized partition; EP—epithelium; GC—gastric caecum; I—intima; IBL—inner barbated lobe; LD—lateral denticle; LM—longitudinal muscle; LT—lateral tooth; MD—median denticle; MT—median tooth; N—neck of proventriculus; OBL—outer barbated lobe; Oes. V.—oesophageal valve; T—sclerotized appendage.

FIG. 44.   Cycloptilum squamosum *Scudder—sclerotized intima of one longitudinal fold of proventriculus.*
FIG. 45.   Myrmecophila oregonensis *Brunner— sclerotized intima of one longitudinal fold of proventriculus.*
FIG. 46.   Gryllotalpa hexadactyla *Perty—sclerotized appendage of proventriculus.*
FIG. 47.   Anurogryllus muticus *DeGeer—sclerotized appendage of proventriculus.*
FIG. 48.   Neoconocephalus ensiger *Harris—sclerotized intima of two longitudinal folds of the proventriculus.*
  BL—barbated lobe; C—cushion of hairs; CH—loop of hairs; CP—sclerotized partition; CT—sclerotized tooth; IBL—inner barbated lobe; LD—lateral denticle; LL—lateral lobe; LT—lateral tooth; MD—median denticle; MT—median tooth; OBL—outer barbated lobe; Oes. V.—oesophageal valve; T—sclerotized appendage.

FIG. 49.   Neoconocephalus ensiger *Harris—transverse section of proventriculus, through sclerotized appendages.*
FIG. 50.   Conocephalus fasciatus *DeGeer—sclerotized intima of two longitudinal folds of proventriculus.*
FIG. 51.   Conocephalus fasciatus *DeGeer—transverse section through neck of proventriculus.*
FIG. 52.   Conocephalus fasciatus *DeGeer—transverse section through oesophageal valve.*
FIG. 53.   Conocephalus fasciatus *DeGeer—transverse section through sclerotized appendage.*
  BL—barbated lobe; C—cushion of hairs; CH—loop of hairs; CM—circular muscle; CP—sclerotized partition; CT—sclerotized tooth; EP—epithelium; I—intima; LL—lateral lobe; LM—longitudinal muscle; MT—median tooth; Oes. V.—oesophageal valve; T—sclerotized appendage.

FIG. 54.   Atlanticus gibbosus *Scudder—sclerotized intima of one longitudinal fold of proventriculus.*
FIG. 55.   Atlanticus gibbosus *Scudder—sclerotized appendage of proventriculus.*
FIG. 56.   Pholidoptera griseoaptera *DeGeer—sclerotized appendage of proventriculus.*
FIG. 57.   Chelidoptera albopunctata *Goeze—sclerotized appendage of proventriculus.*
FIG. 58.   Neduba carinata *Walker—sclerotized appendage of proventriculus.*
FIG. 59.   Anabrus simplex *Haldemann—sclerotized appendage of proventriculus.*
FIG. 60.   Pediodectes haldemanni *Girard—sclerotized appendage of proventriculus.*
FIG. 61.   Stenopelmatus fuscus *Haldemann—sclerotized appendage of proventriculus.*
FIG. 62.   Hemideina megacephala *Buller—sclerotized appendage of proventriculus.*
FIG. 63.   Cratomelus *sp.—sclerotized appendage of proventriculus.*
FIG. 64.   Hadenoecus puteanus *Scudder—sclerotized appendage of proventriculus.*
FIG. 65.   Tachycines asynamorus *Adelung—sclerotized appendage of proventriculus.*
  BL—barbated lobe; C—cushion of hairs; CH—loop of hairs; CP—sclerotized partition; CT—sclerotized tooth; LL—lateral lobe; LP—lateral projection; MT—median tooth; Oes. V.—oesophageal valve.

Fig. 66. Ceuthophilus maculatus *Harris—sclerotized intima of proventriculus.*
Fig. 67. Scudderia curvicauda *DeGeer—transverse section through neck of proventriculus.*
Fig. 68. Scudderia curvicauda *DeGeer—transverse section through a sclerotized appendage of proventriculus.*
Fig. 69. Scudderia curvicauda *DeGeer—sclerotized intima of one longitudinal fold of proventriculus.*

BL—barbated lobe; CC—cushion of hairs; CH—hairs; CM—circular muscle; CP—sclerotized partition; CS—sclerotized spines; .CT—sclerotized tooth; EP—epithelium; I—intima; MT—median tooth; Oes. V.—Oesophageal valve.

Fig. 70. Dissosteira carolina *Linnaeus—sclerotized intima of proventriculus and posterior end of crop.*
Fig. 71. Dendrotettix quercus *Packard—sclerotized intima of proventriculus and posterior end of crop.*
Fig. 72. Chloealtis conspersa *Harris—sclerotized intima of proventriculus and posterior end of crop.*
Fig. 73. Cyphoderris monstrosa *Uhler—sclerotized appendage of proventriculus.*
Fig. 74. Cyphoderris monstrosa *Uhler—sclerotized appendage of proventriculus, lateral view.*

LL—lateral lobe; LP—longitudinal plate of proventriculus; LR—longitudinal ridge of crop; MT—median tooth; S—scalelike spine of crop.

Fig. 75. Dissosteira carolina *Linnaeus—transverse section of crop.*
Fig. 76. Dissosteira carolina *Linnaeus—transverse section of crop anterior to proventricular plates.*
Fig. 77. Dissosteira carolina *Linnaeus—transverse section of proventriculus through anterior ends of proventricular plates.*
Fig. 78. Dissosteira carolina *Linnaeus—transverse section of proventriculus through posterior end of proventricular plates.*
Fig. 79. Tettigidea lateralis parvipennis *Harris—longitudinal section of proventriculus, posterior end of crop, and of anterior end of mid-gut.*
Fig. 80. Paratettix cucullatus *Burmeister—sclerotized intima of proventriculus and posterior end of crop.*
Fig. 81. Cylindroryctes spegazzini *Giglio-Tos- sclerotized intima of proventriculus and posterior end of crop.*
Fig. 82. Tridactylus apicalis *Say- -sclerotized intima of proventriculus and crop.*

C—crop; CM—circular muscle; EP—epithelium; GC—gastric caecum; H—head; I—intima; LF—longitudinal fold of proventriculus; LM—longitudinal muscle; LR—longitudinal ridge of crop; MG—mid-gut; Oes. V.—oesophageal valve; P—proventriculus; TM—transverse muscle.

Fig. 83. Tridactylus apicalis *Say—transverse section of proventriculus and gastric caeca.*
Fig. 84. Termopsis angusticollis *Hagen—sclerotized intima of proventriculus.*

C—cushion; CM—circular muscle; CT—sclerotized tooth; EP—epithelial layer; GC—gastric caecum; I—intima; LF—longitudinal fold; Oes. V.—oesophageal valve.

Fig. 85. Grylloblatta campodeiformis *Walker—sclerotized intima of proventriculus.*
Fig. 86. Grylloblatta campodeiformis *Walker—transverse section through primary folds of proventriculus.*
Fig. 87. Grylloblatta campodeiformis *Walker—transverse section through primary and secondary folds of proventriculus.*
Fig. 88. Grylloblatta campodeiformis *Walker—longitudinal section through proventriculus and anterior end of mid-gut.*

CM—circular muscle; EP—epithelium; GC—gastric caecum; I—intima; LM—longitudinal muscle; N—neck of proventriculus; Oes. V.—oesophageal valve; PF—primary fold; PT1—pyramidal tooth (first row); PT2—pyramidal tooth (second row); S—scalelike spines; SF—secondary old.

Fig. 89. Grylloblatta campodeiformis *Walker—transverse section through primary and secondary pyramidal teeth of proventriculus.*
Fig. 90. Grylloblatta campodeiformis *Walker—transverse section through one primary and two secondary pyramidal teeth of proventriculus.*
Fig. 91. Grylloblatta campodeiformis *Walker—transverse section through secondary pyramidal teeth of proventriculus.*

FIG. 92.   Grylloblatta campodeiformis *Walker—transverse section through one secondary pyramidal tooth of proventriculus.*

FIG. 93.   Acroneuria abnormis *Newman—longitudinal plate of proventriculus.*

FIG. 94.   Acroneuria abnormis *Newman—sclerotized intima of proventriculus.*

CH—hairs; CM—circular muscle; EP—epithelium; I—intima; LM—longitudinal muscle; LT—longitudinal plate; PT1—pyramidal tooth (first row); PT2—pyramidal tooth (second row).

FIG. 95.   Acroneuria abnormis *Newman—transverse section through posterior end of longitudinal plates of proventriculus.*

FIG. 96.   Acroneuria abnormis *Newman—transverse section through posterior end of proventriculus.*

FIG. 97.   Anisolabis maritima *Buller—proventriculus, posterior end of crop, and anterior end of mid-gut.*

FIG. 98.   Anisolabis maritima *Buller—sclerotized intima of proventriculus.*

C—crop; CC—cushion of hairs; CM—circular muscle; EP—epithelium; LF-- longitudinal fold; LM—longitudinal muscle, LT—longitudinal plate; MG —mid-gut; Oes. V.—oesophageal valve; P—proventriculus.

1   1 cm.

2   2 mm.

3   2 mm.

4   1 mm.

5.   2 mm.

6.   2 mm.

13.

PF
SF
CT
Ss
S
AC
PC

0·5 mm.

OesV

14

EP
I
CM
LM
AC

S
CT
H

OesV.

MG

0.5 mm.

CM
PF
LM
RM
EP
H

0·2 mm.

15

0·25mm

PF
CT
OP

S

C

16

Oes.V

1mm

CT
P
PF

AC

PC

17

OesV

18

CT

PF
SF

AC
PC
SC

Imm

Oes.V.

CT

SF
PF

AS
AC
PS

PC

Oes.V.

19

0·5 mm

AR
CT
CC
AC
PC
Oes.V.
0.7mm.
20.

21.
EP
CM
LM
CT
0.74mm.

23.
AC
CH
EP
LM
CM
0.74mm.

22.
FT
R
EP
0.18mm.

26.

S

LF

0 6mm

27.

S

LF

C

I

CM

EP

Inv.

LM

Oes.V.

MG

FL

24

0.7mm.

28.

CT

CC

BL

LL

MT

CP

Oes.V.

C

LF

P

25.

Oes.V.

0.25cm.

0.5mm.

29.

LD
MT
EP
IBL
CP
OBL
LM
CM

0·19 mm.

30.

MD
MT
LD
IBL
CP
OBL
LM
CM

0·19 mm.

31.

LT
LD
EP
CP
OBL
LM
CM

0·19 mm.

32

CL

CA

OBL

CP

Oes.V.

0·35 mm.

33

LT
IBL
MT
OBL
MD

CP

0·1 mm.

34.

LT
IBL
OBL
MT
MD

0·12 mm.

35.

LT
IBL
OBL
MT
MD

0·08 mm.

36.

N

CM

EP

MD

MT

LM

I

Oes.V.

GC

0·5mm.

37.

LT
LD
IBL
MD

0.32mm.

38.

LT
LD
IBL
MT
MD

0.18mm.

39.

LT
LD
IBL
MT

MD

0.18mm.

40.

LT
LD
MT
IBL
MD

0.14mm.

0·5mm.

LT
OBL
IBL
LD
MT
MD

42.

0·08mm.

LT
OBL
LD
IBL
MT
MD

43.

CC

CP

OBL

T

41.

0.7mm.

44

0·2mm

OBL
MT
LT
MD
IBL
LD
CP

OesV

46

LT
LD
MT
IBL
MD

0·25mm

47

LT
LD
MT
IBL
MD

LT
OBL
LD
MT

CP
OesV

0·1mm.

45

48

CT
CH
C
T
MT
LL
BL
CP

Oes.V.

0·7mm

49.

CM
CP
BL
LM
EP
LL
MT
I

0.4 mm.

50

CT
C
CH

T
MT
LL
CP
BL

Oes.V.

0.35mm.

51.

CM
LM
EP
I

0.19 mm.

52

I
EP
LM
CM

0.19 mm.

53

I
MT
EP
LL
BL
LM
CP
CM

0.19 mm.

0 5mm — CT

CH

C

BL

MT

LL

CP

54

Oes.V.

0·25mm. — 55.
LL
LP
MT

0·12mm. — 56.
LL
LP
MT

0·12mm. — 57
LL
LP
MT

012mm. — 58
LL
LP
MT

0·25mm — 59
LL
MT

60   0 6mm
LL
MT

61   01mm.
LL
MT

62
LL
MT
0·25mm.

0 25mm — 63
LL
MT

0·25mm — 64
LL
MT

0 25mm — 65
LL
MT

66.

CT
CC
MT
LL
BL
CP
Oes.V.
0.7mm.

67

CM
EP
LM
CS
0.2mm.

68

CS
EP
LM
LL
BL
CP
CM
0.1mm.

69

CT
CH
BL
CP
LL
MT
CS
Oes.V.
0.4mm.

S

LR

1mm.

LP

70.

S

LR

LP

71.

1mm.

S

LR

72.

LP

MT

74.

1mm.

0.25mm.

LL

73.   MT

0.3mm.   LL

75

CM
LR
LM
EP
I

0.42mm.

76.

CM
LR

0.42mm.

77.

CM
EP
LM
LP

0.42mm.

78.

LP
CM
TM
LM
I
EP

0.42mm.

79.

I
EP
GC
CM
Oes.V.
MG

0.5mm.

80.

LF

1mm.

81.

C
P
Oes.V.

0.3mm.

82.

1mm.
H
C
I

89.

PT2

PTI

CM

0·2mm.

93.

0.74mm.

90.

CH

PTI

0·1mm.

PT2

I

EP

LM

CM

91

CM

PT2

0·3mm.

LT

94

1·4mm.

92.

0·1mm.

CH

EP

LM

I

CM

95.

LM

CM

EP

0.37mm.

96.

LM
LT
CM

EP

0.18mm.

97.

C

P

MG

1mm.

CC

LF

98.

Oes.V.

0.5mm.

THE USE OF RADIOPHOSPHORUS, P³², TO MEASURE PHOSPHORUS UTILIZATION BY LAYING HENS[1]

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C. C. Lee,[5] and Marguerite Reade[5]

Abstract

Phosphorus utilization in four laying hens has been studied by means of radio-phosphorus (P³²), which was incorporated into the laying mash as tricalcium phosphate replacing the bone meal in the diet. The results obtained indicate that, for these four hens:

1. The phosphorus that was absorbed appeared in the yolk, white, and shell within 24 hr. after feeding.

2. The maximum recovery of P³² following a single feeding was within 24 hr. in the case of the shell, 48 to 72 hr. for the white, and 144 hr. (six days) for the yolk.

3. A large portion of the unabsorbed phosphorus was excreted within 24 hr. of feeding.

4. A considerable quantity of the phosphorus absorbed by the digestive system was found to be stored in the tibiae at least 40 days after feeding.

5. The percentage uptake of phosphorus from tricalcium phosphate rose gradually in the egg and became relatively constant in about 14 to 15 days after the first feeding of tricalcium phosphate.

Introduction

It is well known that radiophosphorus, P³², is a valuable tracer tool in studying phosphorus metabolism. A number of studies have already been made with hens. Hevesy and Hahn (7) injected radioactive disodium hydrogen phosphate into laying hens, which were killed some hours later. P³² was determined in various organs and in the egg and it was concluded that the bulk of the phosphatides in the yolk originated in the liver.

Entenman *et al.* (6) injected P³² as disodium hydrogen phosphate and killed the birds 12 hr. later. Eighteen yolks from four birds all contained a small amount of radiophosphorus.

Chargaff (3) injected radioactive sodium phosphate into laying hens and examined the phosphorus compounds in the yolks of the eggs for eight days

following. The activity in the yolk increased to a maximum at the fifth day and then fell off.

Lorenz *et al.* (9) made a detailed study of the fate of P[32] administered subcutaneously as sodium hydrogen phosphate. They found up to 0.59% of the P[32] in the shell six hours after injection. The P[32] reached a maximum of 2.5% in the yolk after about 130 hr. and a maximum of 0.2% in the albumen after about 60 hr.

Scott and others (5, 11) fed P[32] as phosphoric acid and found about 23% excreted in a 60 day period. P[32] was deposited mainly in the musculature and the bones with a shift from the former to the latter with time.

While the above experiments yield a great deal of information the method of administering phosphorus does not correspond very closely to that ordinarily practised in poultry feeding. Bone meal is a common source of phosphorus in poultry rations. Consequently it was thought that useful information might be obtained by replacing the bone meal in the mash with calcium phosphate containing P[32].

The P[32] emits beta particles, which can be counted using a Geiger Counter, the rate of counting giving a measure of the P[32] present and therefore of the original radioactive phosphate and the inactive phosphate with which it was originally mixed. Thus from the measured activity of say, the yolk, the percentage of phosphorus, in the yolk, that came from the added calcium phosphate in the feed, can be calculated. Naturally, due allowance must be made for self absorption of electrons by the material itself and for the decay of the P[32] (half life 14.3 days) (8, pp. 82-94).

Experimental

Outline of Trials

Three birds from the University flock, all in laying condition, were fed a laying mash that had the following composition:

Ground wheat	20.0 lb.
Ground oats	25.0
Ground barley	20.0
Wheat bran	8.0
Wheat shorts	10.0
Meat meal (55%)	7.0
Whey powder	4.0
Vitagras	1.6
Bone meal	1.0
Limestone powder	3.0
Salt	0.5
Fish oil (1200 A/gm., 200 D/gm.)	1.0
Manganese sulphate	¼ lb./ton of mash

Throughout the trials, the bone meal was replaced with calcium phosphate containing P[32]. To prevent loss of radioactivity through spillage, the mash was fed in a moistened condition. Each bird received 2 oz. of this wet mash and 2 oz. of whole grain (wheat and oats) daily. They also had access to a soluble calcium-bearing grit.

The first bird, a Barred Plymouth Rock hen, aged about 14 months, was isolated and fed daily doses of 700 mgm. of radioactive tricalcium phosphate in the mash, for four successive days (June 29 to July 2). All eggs laid after June 29 were hard boiled, separated into the yolk, white, and shell, wet ashed, and the radioactivity and total phosphorus determined. This bird was killed on Aug. 14. The left tibia was removed, cleaned of all adhering tissue, and wet ashed for the quantitative determination of P^{32}.

Both the second and third birds were New Hampshire pullets, aged about five months. The second bird received a single feeding of 700 mgm. of active calcium phosphate mixed in the laying mash on Aug. 17. All eggs laid after this date were hard boiled, separated into the yolk, white, and shell, wet ashed, and the radioactivity and total phosphorus determined. In addition, 24 hr. samples of droppings from the bird were collected in an enamel tray. The droppings were wet ashed and the total phosphorus and radioactivity determined.

The third bird was fed a daily dose of 700 mgm. of calcium phosphate for a period of 25 days (Aug. 12 to Sept. 5). The weight of active calcium phosphate in each 700 mgm. sample was adjusted so that the bird received the same amount of radioactivity every day, more active calcium phosphate being used as time went on to allow for the decay of P^{32} (see Fig. 1). The following is a record of the 25 calcium phosphate feedings:

Date P^{32} fed	Wt. of active $Ca_3(PO_4)_2$, mgm.	Wt. of inactive $Ca_3(PO_4)_2$, mgm.	Total weight, mgm.
Aug. 12	100	600	700
13	105	595	700
14	110	590	700
15	115	585	700
16	121	579	700
17	127	573	700
18	133	567	700
19	140	560	700
20	146	554	700
21	154	546	700
22	161	539	700
23	168	532	700
24	177	523	700
25	185	515	700
26	195	505	700
27	205	495	700
28	215	485	700
29	225	475	700
30	235	465	700
31	248	452	700
Sept. 1	260	440	700
2	274	426	700
3	287	413	700
4	303	397	700
5	317	383	700

Every second egg laid in this 25-day period was broken into a 400 cc. beaker and wet ashed for the determination of radioactivity and total phosphorus.

Preparation of Radioactive Calcium Phosphate

P^{32}, in the form of disodium hydrogen phosphate, was added to a suitable amount of disodium hydrogen phosphate solution. This was added to a slightly acid solution containing the calculated quantity of calcium nitrate.
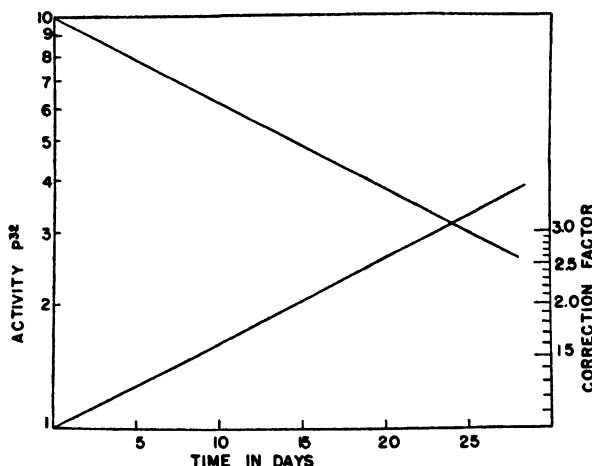
FIG. 1. *Upper curve, activity of P^{32} plotted on semilog paper (left ordinate) against time in days; lower curve, correction factor (right ordinate) to allow for decay of P^{32}. (Example: correction factor after one-half life, 14.3 days, is 2.)*

The solution was heated and calcium phosphate precipitated by the addition of ammonium hydroxide. The precipitate was washed and dried. The total phosphorus was determined and corresponded to that expected for calcium phosphate. The activity was determined using a Geiger-Müller Counter.

Method of Counting

A platinum dish containing the material to be counted was placed in a machined brass plate and slid under the window of a Geiger-Müller beta chamber having a thin mica window (3 mgm. per sq. cm.) at one end. Care was taken to ensure standard geometry. The chamber was connected to a scale of 128 scaling circuit and any given sample was counted for a sufficient length of time to give approximately 10,000 counts (probable error is then approximately 1%) (13). The background count was subtracted from the observed count. Changes in counter efficiency were allowed for by 'sandwiching' the unknown between counts of a uranium oxide standard. In some experiments, e.g. with bird No. 1, the decay of P^{32} was allowed for mathematically using the half life of 14.3 days (Fig. 1).

Activity of Calcium Phosphate Containing P^{32}

Self absorption by the calcium phosphate was allowed for by determining the activity of various weights of active calcium phosphate, spread uniformly in a small platinum dish (2.2 cm. diameter) (see Table I). Plotting the specific activity against the weight allows one to determine the specific activity for

TABLE I

ACTIVITIES OF VARIOUS WEIGHTS OF CALCIUM PHOSPHATE—JULY 29

Counts per minute	Weight, gm.	Counts per minute per mgm.	Correction factor
1560	0.0448	34.8	1.05
2042	0.0613	34.0	1.07
3060	0.0922	33.2	1.10
Extrapolated value for zero weight		36.5	1.00

zero sample thickness (Fig. 2). All counts were corrected to a uranium standard of 3200 counts per minute. The correction curve (Fig. 2) obtained from the last column of the following table agrees very closely with that for magnesium pyrophosphate (13).
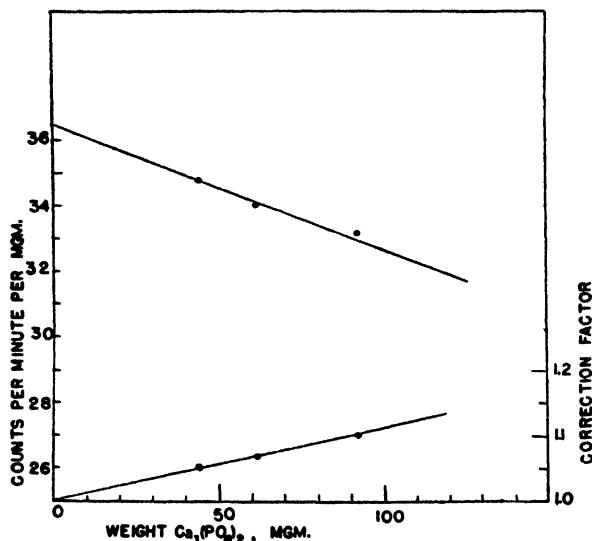
FIG. 2. *Upper curve, activity of sample of calcium phosphate in counts per minute per mgm. (left ordinate) plotted against weight of sample in mgm.; lower curve, correction factor to convert found activity to activity for sample of negligible thickness.*

For the samples from bird No. 2 (which received only a single feeding of P[32]), the decay in the radioactivity was allowed for by 'sandwiching' each sample with a phosphorus standard containing a known weight of the original calcium phosphate. In the repeated dose experiment, the radioactive decay was already allowed for in the daily feeding of calcium phosphate, and therefore a phosphorus standard was not required. In this experiment counter variations were taken care of by sandwiching each sample with a uranium oxide standard. The results were calculated as the percentage uptake of the daily activity fed to the bird. A correction factor was applied to allow for

the decay in the period between the laying of the egg and the determination of its activity. Corrections for self absorption by the sample were applied (13, 8).

Activity of Repeated Dose Experiment

On Aug. 27, 17.4 mgm. of active calcium phosphate had an activity of 4860 counts per minute when the uranium oxide standard was 3108 counts per minute. Hence the activity fed on this date (205 mgm.), corrected to a uranium oxide standard of 3200 counts per minute,

$$= \frac{4860 \times 1.01 \times 3200 \times 205}{17.4 \times 3108} = 59,500 \text{ counts per minute.}$$

Similarly, on Sept. 4, 19.2 mgm. of calcium phosphate had an activity of 3620 counts per minute and the uranium oxide standard had 3040 counts per minute. The activity fed on this date (303 mgm.), corrected to a uranium oxide standard of 3200 counts per minute,

$$= \frac{3620 \times 1.01 \times 3200 \times 303}{19.2 \times 3040} = 61,000 \text{ counts per minute.}$$

The average $\left(\dfrac{59,500 + 61,000}{2}\right) = 6\ 02 \times 10^4$ counts per minute, is there-

fore the amount of activity fed to the bird on each of the 25 days. (With a counter efficiency of 25%, the total activity fed may be calculated to be approximately $3\mu c.$ (microcuries).)

Wet Ashing

The sample (yolk, white, shell, or the whole egg) was digested in 30 to 50 cc. of concentrated nitric acid on a hot plate for two to three hours. This brought the sample into solution except for a thin layer of fatty material observed in the yolk or the whole egg. Ten to fifteen cc. of concentrated sulphuric acid were then added to the yolk and the white, while about 10 cc. of perchloric acid were added to the shell and the whole egg. On continued heating, most of the nitric acid was boiled off and the solution turned black. This could be cleared by the addition of a small amount of concentrated nitric acid. By repeating the process of clearing the blackened solution with nitric acid, eventually all coloring matter is destroyed and a water white solution is obtained. To hasten the clearing process for the yolks and the whites, about 10 cc. of perchloric acid may be added in addition to the 10 to 15 cc. of concentrated sulphuric acid.

Droppings were dried and treated in the same manner as whole eggs.

Total Phosphorus

Total phosphorus was determined colorimetrically, on an aliquot of the wet ash solution, by the hydrazine sulphate method (12).

Determination of Radioactivity

The colorless solution from the wet ashing was diluted to 100 cc. and filtered. An aliquot was taken out, made basic to *p*-dinitrophenol with ammonium

hydroxide and then an equal volume of $2.5N$ nitric acid was introduced. On addition of 10 cc. of ammonium molybdate solution* the yellow ammonium phosphomolybdate precipitated. This precipitate was digested at about 60° C. for 30 min. before it was filtered, dissolved in $6N$ ammonium hydroxide solution and precipitated by 10 cc. of magnesia mixture** plus 5 to 10 cc. of concentrated ammonium hydroxide solution. After digestion at room temperature overnight the magnesium ammonium phosphate was collected by centrifuging and transferred to a platinum dish on which it was dried and ignited to magnesium pyrophosphate and weighed before the sample was counted under the Geiger-Müller counter.

Self-Absorption by the Precipitate

Self-absorption by the magnesium pyrophosphate precipitate was allowed for as in (13). Actually, the correction factor is the same as for calcium phosphate as in Fig. 2.

Results

Bird No. 1

Typical calculation: egg No. 4, laid July 4.

Yolk wet ashed and solution made up to 100 cc. Phosphate from 50 cc. aliquot converted to magnesium pyrophosphate (162 mgm.) and counted July 16 at 1 p.m., 328 counts per minute. Correction factor for self-absorption $= 1.17$, uranium standard 3310 counts per minute.

Total counts per minute based on a uranium standard of 3200 counts per

minute is $\dfrac{328 \times 1.17 \times 3200 \times 2}{3310} = 744$ counts per minute.

Four feeds of calcium phosphate, each 0.7 gm., activity $= 81$ counts per minute per mgm. as of 9 a.m. July 11. Total $= 2.27 \times 10^5$ counts per minute. Correction factor to allow for decay by July 16, 1 p.m. given by

$$\log \frac{n_0}{n} = \frac{0.693t}{2.3 \times 14.3} = \frac{0.693 \times 5.17}{2.3 \times 14.3} = 0.1089$$

$$\text{and } n = \frac{n_0}{1.288} = \frac{2.27 \times 10^5}{1.288} = 1.76 \times 10^5$$

$$\text{and recovery of phosphorus in yolk} = \frac{744 \times 100}{1.76 \times 10^5} = 0.42\%.$$

The percentage recovery of P^{32} from the shell, white, and yolk of eggs laid by bird No. 1 is given in Table II and Fig. 3.

The percentage recovery of P^{32} from the left tibia of this hen 42 days after feeding was 6.7%.

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* *The ammonium molybdate solution was prepared by dissolving 90 gm. of ammonium molybdate in 100 cc. of ammonium hydroxide solution, adding 240 gm. of ammonium nitrate and diluting to a total volume of 1 liter.*

** *The magnesia mixture was prepared by dissolving 50 gm. of magnesium chloride and 100 gm. of ammonium chloride in 500 cc. of distilled water made slightly ammoniacal with ammonium hydroxide. This was allowed to stand overnight, filtered, made slightly acid, and diluted to 1 liter.*

TABLE II

RECOVERY OF P³² LAID BY BIRD No. 1

Egg No.	Egg laid		Recovery of total P³² fed, %			Total P, mgm. (colorimetric)		
	Date	Time	Shell	White	Yolk	Shell	White	Yolk
1	June 30	11:30 a.m.	0.064	0.006	0.001	6.8	3.8	Lost
2	July 1	Noon	0.055	0.018	0.054	7.0	3.5	106.0
3	July 2	4:30 p.m.	0.125	0.025	0.180	9.3	3.8	106.0
4	July 4	11:30 a.m.	0.051	0.032	0.420	8.3	4.1	116.2
5	July 5	3:00 p.m.	0.026	0.036	0.510	6.8	4.5	120.0
6	July 6	3:45 p.m.	0.010	0.008	Lost	8.3	3.8	107.3
7	July 8	11:00 a.m.	*	*	0.398			124.5
8	July 9	6:30 p.m.			0.356			121.5
9	July 10	2:00 p.m.			0.234			114.8
10	July 11	4:30 p.m.			0.161			121.5
11	July 13	11:00 a.m.			0.130			123.0
12	July 14	11:00 a.m.			0.127			131.3
14	July 17	12 noon			0.080			121.5
16	July 20	3:30 p.m.			0.040			117.0
18	July 23	3:00 p.m.			0.040			117.0

* No measurable activity.
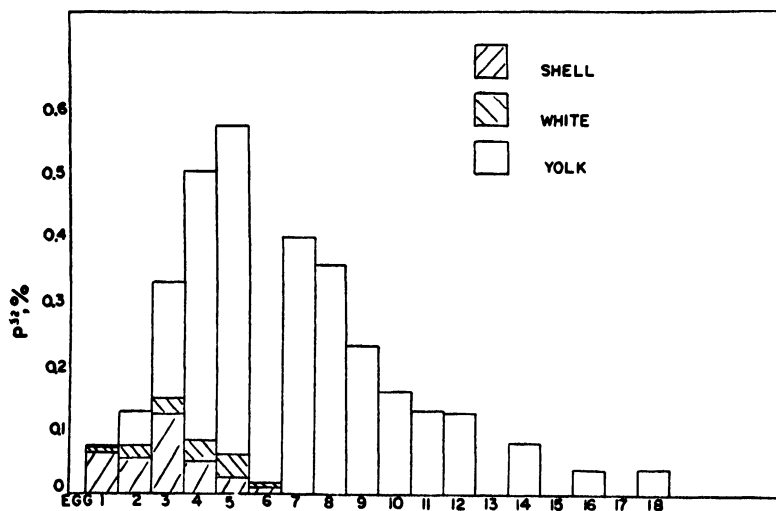
Fig. 3. *Histogram showing recovery of P³² in shell, white, and yolk of successive eggs, expressed as a percentage of the total P³² fed. P³² fed as active calcium phosphate on four successive days.*

Bird No. 2

Typical calculation: egg No. 3, yolk. Phosphorus converted to magnesium pyrophosphate, activity 820 counts per minute.

Sample weight = 77.6 mgm. Therefore correction factor (Fig. 2) = 1.08.

Standard phosphate sample weight 19.2 mgm., activity 3604 counts per minute; correction factor $= 1.01$.

$$\text{Uptake of } P^{32} = \frac{820 \times 1.08}{3604 \times 1.01} \times \frac{19.2}{700} \times 100 = 0.66\% .$$

The following is the weight and date of eggs laid by bird No. 2:

Egg No.	Date of laying	Weight of egg, gm.
1	Aug. 19	53
2	21	50
3	22	50
4	24	50
5	26	50
6	28	51
7	30	54
8	Sept. 1	53
9	3	54
10	5	57
11	6	54

The recovery of activity from the various parts of the eggs laid by bird No. 2 is given in Table III and Fig. 4.

TABLE III

EGGS FROM BIRD NO. 2, PERCENTAGE UPTAKE OF PHOSPHORUS FROM CALCIUM PHOSPHATE

Egg number and part		Uptake of P^{32}, %	Total P, mgm.
Yolk No.	1	0.05	72.0
	2	0.25	73.0
	3	0.66	77.0
	4	0.95	73.0
	5	—*	—*
	6	0.64	94.8
	7	0.35	87.2
	8	0.19	88.0
	9	0.12	88.0
	10	0.13	96.0
	11	0.12	90.0
White No.	1	0.08	3.2
	2	0.16	3.4
	3	0.07	4.3
	4	0.06	4.0
	5	0.02	4.0
	6	0.22	5.0
	7	0.04	5.0
Shell. No.	1	0.36	5.2
	2	0.13	7.6
	3	0.05	6.2
	4	0.89**	7.0
	5	0.07	6.9
	6	0.02	7.5
	8	0.02	6.8

* *Lost on ashing.*
** *Probably contaminated during ashing.*
Whites, Nos. 8 to 11, and shells, Nos. 7, 9 to 11, had no measurable activity.
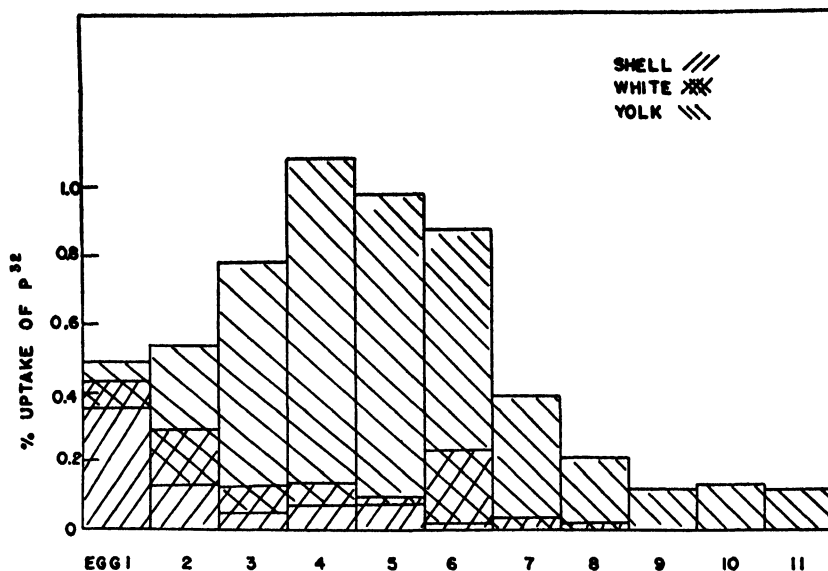
FIG. 4. *Histogram showing recovery of P³² in shell, white, and yolk of successive eggs,
expressed as a percentage of the total P³² fed. P³² fed as a single feeding of active calcium
phosphate.*

The total phosphorus and the percentage of P³² excreted in each days'
droppings for bird No. 2 are shown in Table IV.

TABLE IV

PERCENTAGE PHOSPHORUS FROM CALCIUM PHOSPHATE APPEARING
IN DROPPINGS—BIRD No. 2

Date of droppings	Excretion of P³², %	Total P, mgm.
Aug. 18	15.90	552
19	2.39	420
20	1.92	450
21	1.03	480
22	0.63	400
23	0 44	528
24	0.21	397
25	0 20	429
26	0.07	461
27	0.50	528
28	0.18	395
29	0.06	502
30	0.00	415

Since it was possible that there might have been some contamination of the
droppings of Aug. 18 and 19, the experiment was repeated with bird No. 4.
These results are recorded in Table V.

TABLE V

PERCENTAGE PHOSPHORUS FROM CALCIUM PHOSPHATE APPEARING IN DROPPINGS—BIRD No. 4

Date of droppings	Excretion of P^{32}, %	Date of droppings	Excretion of P^{32}, %
Sept. 26	8.66	Sept. 28	0.58
Sept. 27	0.74	Sept. 29	0.00

Bird No. 3

Typical calculation: egg No. 9, laid Aug. 23.

Counted Sept. 4, total activity 1098 counts per minute.*

Sample weight = 68.4 mgm. Correction factor for self-absorption by sample is 1.06.

Decay factor, 12 days, is 1.77.

Daily activity fed = 60,200 counts per minute (see page 168)*.

$$\% \text{ recovery} = \frac{1098 \times 1.06 \times 1.77}{60,200} \times 100 = 3.42 \ .$$

The percentage uptake of phosphorus for bird No. 3 is shown in Table VI and Fig. 5.

TABLE VI

EGGS FROM BIRD No. 3, PERCENTAGE UPTAKE OF PHOSPHORUS
(REPEATED DOSE EXPERIMENT)

Egg number	Date of laying	Weight of egg, gm.	Uptake of P^{32}, %	Total P, mgm.
1	Aug. 12	40	0.25	71.5
2	15	45	0.86	68.0
4	17	42	2 66	72.5
5	19	46	3.16	77.5
7	21	50	3.98	84.5
9	23	50	3.42	100.0
11	25	46	4.34	90.0
13	27	48	3.78	104.8
15	30	52	3.83	104.0
17	Sept. 1	45	3.76	104.0
19	4	47	3 55	96.0

Discussion

In these experiments, P^{32} was fed either in repeated doses or as a single dose in order to determine the fate of phosphorus when fed to laying birds. By referring to Table III, it will be seen that phosphorus is deposited in the egg within 24 hr. of feeding. A large proportion of this phosphorus is located in the shell. It is interesting to note that the percentage recovery in the shell decreases sharply after the first feeding. The amount of P^{32} recovered from

* *Corrected to a uranium oxide standard of 3200 counts per minute.*

the yolk and white within the first 24 hr. after feeding is relatively small since these components of the egg are almost completely formed some 20 hr. before laying (14).   As each succeeding egg is laid, the total amount of P^{32} recovered
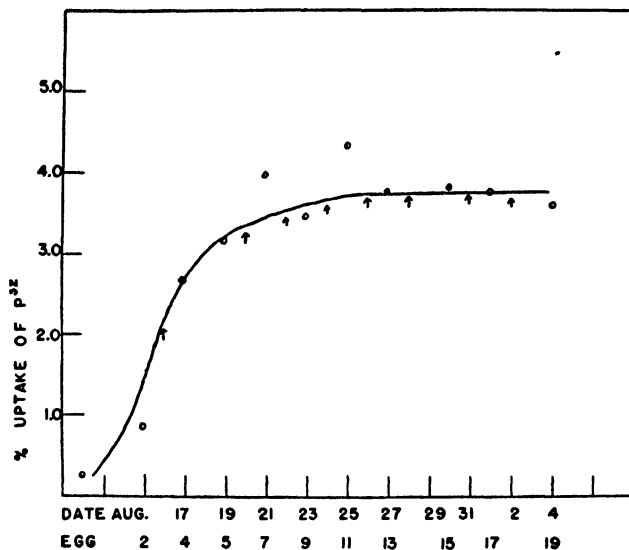
FIG. 5.   *Recovery of P^{32} in successive whole eggs expressed as a percentage of the P^{32} fed on any one day.   Same activity of P^{32} fed as active calcium phosphate for 25 successive days. (Eggs not analyzed indicated by an arrow.)*

from the yolk increases at a fairly uniform rate.   This is in agreement with the results of Lorenz *et al.* (9) and Chargaff (3).   The fact that the peak of recovery is approximately six days after feeding would indicate that this is the length of time for the yolk to pass through the late stage of greatly accelerated growth.   This substantiates the findings of Riddle (10).   With respect to the white, the maximum recovery was noted within 24 to 72 hr., which is in accord with Lorenz *et al.* (9).

The analyses of the eggs from bird No. 1 (Table II) follow the same general trend but are not so distinct since she was fed four successive doses of P^{32}.

However, the marked break in the curve occurring at egg No. 10 in Fig. 3 is interesting.   It is known that the yolk is laid down in about 10 days and thus an egg laid more than 10 days after the activity was administered could not have obtained its activity directly from the feed but only indirectly, for example, from the tissue and skeletal matter.   This is in agreement with the general idea of 'exchange' of phosphorus compounds within the body and finds support in the fact that the left tibia of bird No. 1 showed 6.7% of the P^{32} fed, 42 days after feeding.

The experiment in which the bird is fed a single dose of labelled tricalcium phosphate shows very clearly that this single dose is eventually distributed through a larger number of eggs.

The phosphorus in any given egg must, of course, come from the phosphorus fed on many different days, and from a material balance, we can say that, for regular feeding and laying, the total phosphorus from the feed appearing in any one egg should equal the total utilization of any one day's feed. The situation is illustrated graphically in Fig. 6. The fate of the phosphorus fed on successive days is shown by the full curves, the total phosphorus obtained on successive days being given by the sum of these curves i.e. by the broken line curve in Fig. 6. It evidently agrees very well with experiment (Fig. 5).
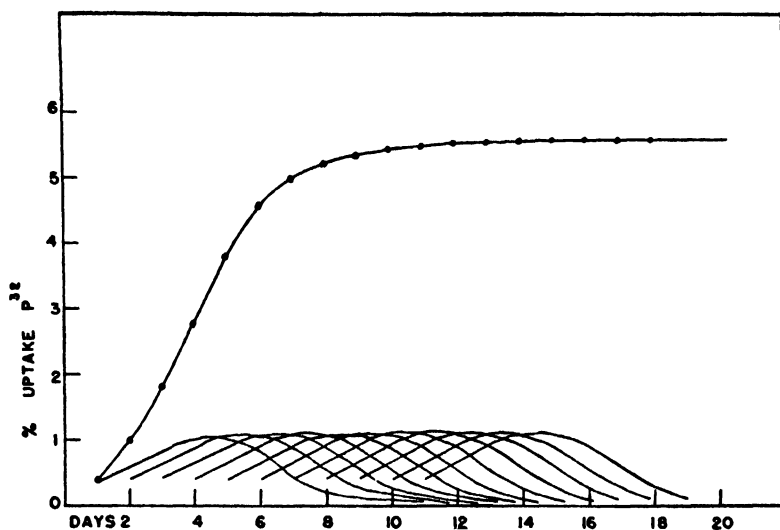
FIG. 6. Theoretical ~~~~~~~ing recovery of P[34] (as percentage of daily P[34] fed) in successive eggs in multiple feeding experiment as a summation of recoveries in successive single feeding experiments.

The percentage uptake of phosphorus from a specific source (in this case active calcium phosphate) rises gradually in the egg (Table VI and Fig. 5) and becomes relatively constant in 14 or 15 days after the first feeding. Cook et al. (5) fed radioactive phosphoric acid to growing birds and studied the percentage uptake of P^{32} in different organs at varying lengths of time after feeding. However, no work seems to have been reported on the time required for tagged phosphorus to reach a constant level in the eggs of birds that are laying at a uniform rate of production.

The ashing of the tibia of bird No. 1 indicated that there is a large amount of phosphorus stored in the bones. Further studies on laying birds similar to that reported by Cook et al. (5) with growing birds should prove worth-while.

The results obtained from the analysis of the droppings of bird No. 2 indicate that the biggest excretion of phosphorus is within 24 hr. after feeding (see Table IV). The amount of excretion of the P^{32} (and this is indicative of the phosphorus excreted that was fed as calcium phosphate) decreases very rapidly after the first 24 hr. following feeding, and is negligible in about 12

days.   The results of ashing the droppings of bird No. 4 (Table V) would indicate an even faster rate of decrease in excretion of phosphorus following the first 24 hr. after feeding.   This may be an individual characteristic of each hen.   Lorenz *et al.* (9) found a marked irregularity in the excretion of P³². Our results are in agreement with those of Common (4) and Lorenz *et al.* (9).

Since the birds were fed ground limestone in the mash and had access to a soluble calcium-bearing grit, the amount of tricalcium phosphate had no apparent effect on egg shell quality as was noted by Buckner *et al.* (1, 2) when they fed precipitated tricalcium phosphate as the only mineral supplement.   These authors concluded that poor egg shells were produced when tricalcium phosphate was fed as the only mineral supplement because calcium was a limiting factor.   This would not be the case in these experiments since both ground limestone and a soluble calcium-bearing grit were fed.

Further investigations with radioactive minerals are in progress at the present time.

Acknowledgments

References

1. BUCKNER, G. D., MARTIN, J. H., and PETER, A. M.   The relative utilization of different calcium compounds in the production of eggs.   J. Agr. Research, 36 : 263-268.   1928.
2. BUCKNER, G. D., MARTIN, J. H., PIERCE, W. C., and PETER, A. M.   Calcium in egg-shell formation.   J. Biol. Chem. 51 : 51-54.   1922.
3. CHARGAFF, E.   The formation of phosphorus compounds in egg yolk.   J. Biol. Chem. 142 : 505-512.   1942.
4. COMMON, R. H.   Observations on the mineral metabolism of pullets. II.   J. Agr. Sci. 26 : 85-100.   1936.
5. COOK, S. F., SCOTT, K. G., and ABELSON, P.   The deposition of radio-phosphorus in tissues of growing chicks.   Proc. Natl. Acad. Sci. U.S. 23 : 528-533.   1937.
6. ENTENMAN, C., RUBEN, S., PERLMAN, I., LORENZ, F. W., and CHAIKOFF, I. L.   Radio-active phosphorus as an indicator of phospholipid metabolism.   III.   The conversion of phosphate to lipoid phosphorus in the laying and non-laying bird.   J. Biol. Chem. 124 : 795-802.   1938.
7. HEVESY, G. and HAHN, L.   The origin of phosphorus compounds in hen eggs.   Kgl. Danska Videnskab. Selskab.   Biol. Medd. 14 : No. 2.   1938.
8. KAMEN, M. D.   Radioactive tracers in biology.   Academic Press, Inc., New York.   1947.
9. LORENZ, F. W., PERLMAN, I., and CHAIKOFF, I. L.   Phosphorus deposition in the egg as measured with radioactive phosphorus.   Am. J. Physiol. 138 : 318-327.   1942-1943.
10. RIDDLE, O.   The formation, significance and chemistry of the white and yellow yolks of ova.   J. Morphol. 22 : 455-491.   1911.
11. SCOTT, K. G. and COOK, S. F.   The effect of radioactive phosphorus on the blood of growing chicks.   Proc. Natl. Acad. Sci. U.S. 23 : 265-272.   1937.
12. SHELTON, W. R. and HARPER, H. J.   A rapid method for the determination of total phosphorus in soil and plant material.   Iowa State Coll. J. Sci. 15 : 403-413.   1941.
13. SPINKS, J. W. T. and BARBER, S. A.   Study of fertilizer uptake using radioactive phosphorus.   Sci. Agr. 27 : 145-156.   1947.
14. WARREN, D. C. and SCOTT, H. M.   The time factor in egg formation.   Poultry Sci. 14 : 195-207.   1935.

TOXICITY OF SELECTED ORGANIC COMPOUNDS TO INSECTS

PART I. TESTS FOR GENERAL TOXICITY ON LARVAE OF *MUSCA*, *TRIBOLIUM*, AND *EPHESTIA*, AND ADULTS OF *SITOPHILUS*[1]

BY A. W. A. BROWN,[2] D. B. W. ROBINSON,[2] H. HURTIG,[2] and B. J. WENNER[2]

Abstract

The general toxicity of 127 synthetic organic compounds was tested against larvae of *Musca domestica*, *Tribolium confusum*, and *Ephestia kuehniella*, and adults of *Sitophilus granarius*. The compounds were mixed in the insects' food in graded concentrations, and their toxicity was assessed by determination of the median lethal concentrations (LC_{50}) for each of the four species. The most highly toxic compounds were gammexane (the gamma isomer of hexachloro-cyclohexane) and chlordane (obtained by distillation of technical chlordane). The toxicity of DDT was on the average one-half of that of the first two compounds, and it was superior to any of the 12 analogues tested. Four chlorinated aliphatic hydrocarbons, namely hexachloropropene, hexachlorobutadiene, and the *symm*-and *asymm*-heptachloropropanes, showed a high toxicity related to their powerful fumigant action. A high level of toxicity was shown by benzyl thiocyanate and its chlorinated derivatives. The nitro compounds dinitro-*o*-cresol, nitrostyrene, dinitrodimethylbutane and dinitrocyclohexylphenol were especially toxic to *Sitophilus* adults, but were ineffective against *Musca* larvae. Certain aromatic semicarbazones recommended by previous workers gave disappointing results. Of 22 derivatives of morpholine tested, only three showed any degree of toxicity to the four species of insects employed.

Introduction

A program of synthesis of selected organic compounds for tests on certain species of insects has been undertaken at the Experimental Station, Suffield, Alta. The 127 compounds that were selected for investigation fell mainly in the following groups: chlorinated aliphatic and cyclic hydrocarbons, cyanides, and nitriles, thiocyanates, derivatives of morpholine, and compounds analogous to DDT. Certain compounds outside these classifications were also included.

This paper covers the initial screening tests for general toxicity of the compounds to four species of insects. In this type of test the compound is intimately mixed with the food medium in which the insect lives and feeds. It thus provides an over-all measure of the contact, stomach, and fumigant toxicity of the compound, without distinction as to the mode of action. The compounds were later tested for contact toxicity to three species of insects (see Part II* of this series). Testing of their stomach toxicity alone was not undertaken.

It was considered that these compounds, which were carefully selected to contain molecular structures that might prove insecticidal, were worthy of a

thorough type of investigation. Accordingly the insects on which they were tested were representative of several orders, namely the Orthoptera, Hemiptera, Lepidoptera, Diptera, and Coleoptera, and examples of larvae and nymphs as well as adults were employed. In this way the possibility of passing over an insecticide by reason of using an unsuitable test insect species or developmental stage was minimized. Moreover it was felt that the data might yield information on the species or group specificity of toxic action of a particular compound or class of compounds.

Throughout these investigations the compounds were tested at a number of concentrations, which were progressively reduced until the resulting mortality was less than 20%. From the percentage mortality figures the median lethal concentration (here treated synonymously with the term LC_{50} as the point at which 50% mortality is expected) and the LC_{90} were calculated for each compound.

Material

The biological material consisted of larvae of the housefly (*Musca domestica* L.), the confused flour beetle (*Tribolium confusum* Duval), and the Mediterranean flour moth (*Ephestia kuehniella* Zeller), and adults of the granary weevil (*Sitophilus granarius* L.). They were taken from stocks continuously reared in the laboratory at 78° F. and 65% relative humidity. The stock of *Musca* was reared according to the standard Peet-Grady technique (8). *Tribolium* was reared on whole wheat flour in open enamel trays, and *Ephestia* was reared on corn meal and whole wheat flour in gauze-topped battery jars. The cultures of *Sitophilus* were kept continuously on hard wheat grains in battery jars.

The larvae of *Musca* employed in the tests were obtained by inoculating newly-laid eggs on the standard rearing medium and incubating them for two days at 78° F. In the case of *Tribolium* and *Ephestia* the adults were confined over sieved household flour, and the larvae were obtained 10 to 14 days later by sieving the flour, when they were 7 to 11 days old. The adults of *Sitophilus* were taken from the culture two to four weeks after emergence.

The organic compounds to be tested had been synthesized or specially purified for the purpose. The majority (93) were produced by Dr. D. B. W. Robinson and Mr. J. B. Reesor of the Experimental Station, Suffield, and are designated by the prefix ESS. Eighteen compounds were submitted by Prof. G. F Wright, Department of Chemistry, University of Toronto, and bear the prefix UT. Eight compounds were obtained from the Department of Chemistry, McGill University, and are denoted by McG. An additional eight compounds were kindly supplied by Mr. C. P. Clausen, Bureau of Entomology and Plant Quarantine, U.S. Department of Agriculture, and are designated by the prefix USDA.

The sample of chlordane (1) was obtained by double distillation of technical chlordane (the Technical 1068 of the Velsicol Corporation). The sample of

isoborneyl thiocyanoacetate was obtained by distillation of the insecticide Thanite of the Hercules Powder Company.

The sample of dinitro-*o*-cresol employed (ESS 62) showed a melting point of 88° C. This compound is termed 4,6-dinitro in the Handbook of Chemistry and Physics and the Dictionary of Organic Compounds (Heilbron & Bunbury), but is usually referred to as 3,5-dinitro in commercial specifications and in current entomological literature.

Methods

The test media were prepared by intimately mixing the compounds with the food in proportions ranging from 0.64% (of the air-dry weight of the food material) down to the lowest dilution of 0.000005% (i.e. 6400 down to 0.05 parts per million). Each successive dilution was one-half of the preceding one, and tests were performed with it if the mortality at the preceding concentration exceeded 20%. In all cases 4 oz. specimen jars with screw tops of copper screening were employed. The compounds were finely ground in a mortar, added to the medium, and mixed thoroughly by revolving the containers on eccentric rollers. For concentrations below 0.2%, the compound was added in acetone solution and the solvent was evaporated during mixing under a blast of hot air from a portable hair-drier.

For *Musca* larvae, the test medium consisted of 23 gm. of bran and alfalfa in the ratio of 2 to 1, to which 30 cc. of an aqueous solution containing 6.7% of malt extract (Bynin) and 0.05% of dried brewer's yeast (Dow N.B.) was added and mixed to form a homogeneously moist mass. Each sample thus prepared was inoculated with 50 young larvae. Two or three replicates were set up for every concentration of each compound. The test samples were placed in an incubator at 80° F., and after three days they were examined and the fresh pupae and live larvae remaining were counted. Control mortality in this medium was found to be 0.2% on the basis of 36 control samples set up parallel with the tests.

For larvae of *Ephestia* or *Tribolium*, the test medium consisted of 20 gm. of whole wheat flour. Each test sample was prepared in duplicate and was inoculated with 20 young larvae. They were held at 80° F. for five to six weeks, and were then examined for mortality. The survivors were in the larval or pupal stages. Parallel control samples were run with each group of tests; the average control mortality for *Tribolium* was 3.7% out of 59 control samples, and for *Ephestia* it was 4.1% out of 49 control samples. One group of tests with *Tribolium* and two with *Ephestia* were discarded owing to high control mortality.

The procedure in which adults of *Sitophilus* were used was based on the method reported by Swingle, Phillips, and Gahan (13). The test medium consisted of 15 gm. of hard wheat grains coated with a known amount of the compound under test. Each sample was inoculated with 25 *Sitophilus*

adults, and the mortality was recorded after a period of four days at 80° F. Tests were performed for the most part in duplicate. The average control mortality, out of 70 control samples, was 3.5%.

Results

The percentage mortalities obtained with successive dilutions of the 127 compounds tested against the four species of insects will not be reported fully. However, the results obtained with the 15 compounds that proved to be most toxic for *Musca* are shown in detail in Table I, to indicate the type of data obtained. No correction was applied to compensate for the low control mortality rate.

TABLE I

Mortality data for thl 15 most toxic compounds tested against *Musca* larvae

Average control mortality: 0 2%

Group	Name of compound	Percentage mortality at the following concentrations in p p m									
		6400	3200	1600	800	400	200	100	50	25	12 5
B	Chlordane	100	100	100	94	100	100	10u	100	60	0
A	s Heptachloropropane	100	100	100	95	95	89	96	65	62	6
B	Gammexane	100	100	100	100	100	100	87	77	12	—
B	Benzotrichloride	100	100	100	94	90	96	88	2	1	—
A	Hexachloropropene	100	100	100	100	100	76	20	3	—	—
B	*p*-Chlorobenzyl chloride	100	100	98	91	86	80	4	—	—	—
B	*o*-Chlorobenzyl chloride	100	100	100	100	62	39	11	9	—	—
L	Benzyl thiocyanate	100	100	100	96	78	27	14	4	—	—
A	*as* Heptachloropropane	100	100	100	93	90	39	5	—	—	—
D	o Chlorobenzyl cyanide	100	100	99	99	45	33	19	—	—	—
D	Phenylacetonitrile	100	100	96	91	88	13	12	—	—	—
D	Phthalonitrile	100	100	100	89	66	17	10	—	—	—
D	Benzonitrile	100	100	100	88	17	21	4	—	—	—
L	o Chlorobenzyl thiocyanate	100	98	100	74	50	22	19	—	—	—
D	2 4-Dichlorobenzyl cyanide	99	98	99	83	20	12	—	—	—	—

The approximate LD_{50} and LD_{90} for each compound was obtained by plotting the logarithm of the percentage mortality by the method of Bliss (2). In practice the concentration and mortality figures were plotted directly on logarithmic–probit graph paper (Winthrop Chemical Co.). The percentage concentrations corresponding to 50 and 90% mortalities could be read directly from the regression lines thus obtained.

The values for the LC_{50} and LC_{90} derived for each of the 127 compounds for the four species employed are tabulated in Table II. The figures are expressed in parts per million, an LC_{50} of 6400, for example, indicating 50% mortality at a concentration of 0.64%. Where the LC_{90} or LC_{50} was in excess of 7000 p.p.m. the symbol "Neg" is used to indicate nontoxicity at that level. The symbol "—" indicates that the compound, due to its scarcity, was not tested against the species concerned.

TABLE II

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC$_{50}$) AND LC$_{90}$ FIGURES OF 127 COMPOUNDS
FOR LARVAE OF *Musca, Tribolium,* AND *Ephestia,* AND ADULTS OF *Sitophilus*.

Expressed in parts per million, by weight

No.	Name of compound	Musca		Sitophilus		Tribolium		Ephestia	
		LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$
Chlorinated aliphatics									
ESS 107	1,1,1-Trichloroethane	Neg	Neg	Neg	Neg	Neg	Neg	5700	Neg
ESS 139	2,3-Dichloropropane	Neg	Neg	3000	Neg	Neg	Neg	5700	Neg
ESS 134	2,3-Dichloropropene-1	2900	5000	Neg	Neg	Neg	Neg	5000	Neg
ESS 83	1,2,3-Trichloropropane	1600	2300	Neg	Neg	3500	5000	1800	3600
ESS 56	Hexachloropropene	140	250	450	950	10	110	4	7
ESS 37	*asymm*-Heptachloropropane	250	580	360	500	30	350	80	230
ESS 100	*symm*-Heptachloropropane	38	100	450	870	400	1300	55	220
ESS 81	Octachloropropane	900	1800	1290	2300	Neg	Neg	200	450
ESS 118	1,4-Dichlorobutane	3300	5200	Neg	Neg	3200	4600	2100	3400
ESS 57	Hexachlorobutadiene-1,3	2800	Neg	2200	3800	15	200	25	300
Halogenated cyclic compounds									
McG 2	*p*-Dichlorobenzene	2800	4400	Neg	Neg	3200	6000	800	2400
ESS 90	Gamma-hexachlorocyclohexane	44	90	0.1	0 25	3	18	10	35
McG 1	*p*-Chlorobenzaldehyde	--	--	1450	2400	Neg	Neg	Neg	Neg
ESS 111	*o*-Chlorobenzyl chloride	220	480	1100	1840	280	4000	1800	3500
ESS 112	*p*-Chlorobenzyl chloride	160	440	3500	Neg	350	2200	1800	2800
ESS 132	2,4-Dichlorobenzyl chloride	620	1500	1250	1900	2200	3500	2300	4000
ESS 133	3,4-Dichlorobenzyl chloride	1000	1850	1300	2200	2600	4200	2100	3900
ESS 109	Benzotrichloride	77	130	1160	1830	500	900	800	1900
ESS 108	Benzotrifluoride	6500	Neg	1850	3050	Neg	Neg	6400	Neg
ESS 74	*p*-Chloroacetophenone	1400	2900	580	1000	1650	2700	2600	4500
ESS 75	*p*-Chloropropiophenone	3200	4600	560	1000	3500	5200	5200	Neg
ESS 110	α-Chloro-1-methylnaphthalene	2300	4200	210	380	4000	5100	1900	3500
UT 6	5-Chlorofurfural	5300	Neg	1500	2700	Neg	Neg	Neg	Neg
UT 7	5-Bromofurfural	7000	Neg	1650	2600	Neg	Neg	Neg	Neg
UT 1	Cholesteryl chloride	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
UT 8	Chlordane	23	34	1.3	4.5	0.2	1.3	36	75
DDT and analogues									
ESS 33	*p,p'*-Dichlorodiphenyltri-chloroethane	700	1600	16	41	16	210	860	2700
McG 3	*p,p'*-Diiododiphenyltri-chloroethane	Neg	Neg	800	Neg	Neg	Neg	Neg	Neg
ESS 69	*p,p'*-Dimethyldiphenyltri-chloroethane	Neg	Neg	1200	2100	Neg	Neg	1600	Neg
McG 6	*p,p'*-Dibromomethyldiphenyl-trichloroethane	7000	Neg	1900	Neg	Neg	Neg	Neg	Neg
ESS 72	*p,p'*-Dimethoxydiphenyltri-chloroethane	Neg	Neg	840	1700	Neg	Neg	500	1800
ESS 71	*p,p'*-Diethoxydiphenyltri-chloroethane	1900	3900	1900	2600	110	5000	450	1800
ESS 70	*p,p'*-Dichlorodiphenyldi-chloroethylene	Neg	Neg	1550	3300	2700	6500	1300	3500
McG 7	Carbinol DDT benzoate	--	--	--	--	--	--	Neg	Neg

TABLE II—*Continued*

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC$_{50}$) AND LC$_{90}$ FIGURES OF 127 COMPOUNDS
FOR LARVAE OF *Musca, Tribolium,* AND *Ephestia,* AND ADULTS OF *Sitophilus*—Continued

Expressed in parts per million, by weight

No.	Name of compound	Musca		Sitophilus		Tribolium		Ephestia	
		LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$
DDT and analogues—Concluded:									
ESS 19	Trichloroethylidene-bis-benzamide	Neg	Neg	2300	4200	Neg	Neg	Neg	Neg
ESS 18	Trichloroethylidene-bis-phenacetamide	Neg	Neg	2900	5600	Neg	Neg	Neg	Neg
UT 12	*p,p'*-Dichlorobenzophenone oxime	Neg	Neg	1300	Neg	Neg	Neg	Neg	Neg
McG 8	bis(Dichlorothienyl)-tri-chloroethane	—	—	1350	2200	Neg	Neg	Neg	Neg
ESS 55	4,4'-bis(N-Benzylanilino)-methane	Neg	Neg	Neg	Neg	Neg	Neg	—	—
Cyanides and nitriles									
ESS 85	Octanoyl nitrile	2100	2700	Neg	Neg	5400	Neg	3100	5000
ESS 86	Decanoyl nitrile	4200	7000	4230	Neg	3800	6500	3700	5000
ESS 87	Tetradecanoyl nitrile	4000	8000	970	1850	Neg	Neg	6000	Neg
ESS 88	Octadecanoyl nitrile	Neg	Neg	1950	3600	Neg	Neg	3300	5000
ESS 1	Benzonitrile	400	750	1700	2500	6500	Neg	800	3800
ESS 7	Benzonitrile hexachloride	Neg	Neg	—	—	—	—	—	—
USDA 2	Phthalonitrile	320	760	360	910	900	4000	770	3500
ESS 2	Phenylacetonitrile	310	850	660	1040	4500	Neg	2000	Neg
ESS 24	*p*-Nitrophenylacetonitrile	2800	Neg	800	1220	5200	Neg	1100	3800
ESS 103	*o*-Chlorobenzyl cyanide	270	650	580	1000	3400	4800	2600	4700
ESS 104	*p*-Chlorobenzyl cyanide	640	1250	200	500	300	2500	3100	4500
ESS 135	2,4-Dichlorobenzyl cyanide	550	960	1100	2000	250	6000	5100	Neg
ESS 137	3,4-Dichlorobenzyl cyanide	750	1100	590	1050	2000	3200	4000	5500
ESS 9	*β*-Phenylpropionitrile	780	1850	940	1350	6000	Neg	Neg	Neg
UT 4	Styryl cyanide	1300	2100	107	250	1100	2100	2400	4000
ESS 101	*α*-Cyano-1-methylnaphthalene	3100	5100	520	970	Neg	Neg	5500	Neg
N-Methyl carbamates:									
ESS 116	of *m*-diethylaminophenol methochloride	Neg	Neg	1600	3500	Neg	Neg	Neg	Neg
ESS 115	of *m*-diethylaminophenol methiodide	Neg	Neg	1500	2900	Neg	Neg	Neg	Neg
ESS 117	of *m*-diethylaminophenol methosulphate	Neg	Neg	1750	3050	Neg	Neg	Neg	Neg
BSS 114	of 2-methyl-5-dimethylamino-phenol methochloride	Neg	Neg	1330	2550	Neg	Neg	Neg	Neg
ESS 113	of 2-methyl-5-dimethylamino-phenol methiodide	Neg	Neg	1450	2800	2500	4000	1400	3400
Semicarbazones:									
USDA 3	of ethyl methyl ketone	—	—	1010	1720	Neg	Neg	3000	4500
USDA 4	of 2-furaldehyde	—	—	1800	3800	Neg	Neg	3300	5000
USDA 5	of cyclopentanone	2700	6600	1300	2100	Neg	Neg	3800	5000

TABLE II—*Continued*

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC$_{50}$) AND LC$_{90}$ FIGURES OF 127 COMPOUNDS
FOR LARVAE OF *Musca, Tribolium*, AND *Ephestia*, AND ADULTS OF *Sitophilus*—Continued

Expressed in parts per million, by weight

No.	Name of compound	Musca		Sitophilus		Tribolium		Ephestia	
		LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$
Semicarbazones:—Concluded									
USDA 6	of 2,4-dimethyl-3-pentanone	—	—	1250	2000	Neg	Neg	Neg	Neg
USDA 7	of cyclohexanone	—	—	1350	2100	Neg	Neg	3000	4500
USDA 8	of *p*-chloroacetophenone	—	—	1350	2000	Neg	Neg	3200	4500
Morpholine compounds									
ESS 10	Morpholine	Neg	Neg	980	1850	5200	Neg	Neg	Neg
ESS 12	N-Ethylmorpholine	Neg	Neg	4150	Neg	Neg	Neg	Neg	Neg
ESS 14	N-Butylmorpholine	5300	Neg	2500	4000	Neg	Neg	230	1350
ESS 73	N-Trichoroacetylmorpholine	Neg	Neg	820	2500	550	5500	Neg	Neg
ESS 15	N-Phenylmorpholine	Neg	Neg	600	1500	3100	Neg	1150	4000
ESS 89	N-(*p*-Chlorophenyl)morpholine	6000	Neg	1000	1280	Neg	Neg	2300	3900
ESS 17	N-Benzoyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 29	N-(*p*-Tolyl)morpholine	Neg	Neg	150	310	3400	Neg	1060	3000
ESS 30	N-(*m*-Tolyl)morpholine	Neg	Neg	260	700	4800	Neg	800	2500
ESS 43	N-(*p*-Chlorobenzoyl) morpholide	Neg	Neg	2000	3500	Neg	Neg	Neg	Neg
ESS 45	N-(*o*-chlorobenzoyl) morpholide	Neg	Neg	990	Neg	Neg	Neg	Neg	Neg
ESS 65	N (*m*-Nitrobenzoyl) morpholide	Neg	Neg	1900	Neg	Neg	Neg	Neg	Neg
ESS 26	Benzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 27	*p*-Bromobenzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 28	*p*-Toluenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 49	Morpholine N-sulphonyl anilide	Neg	Neg	1300	2800	Neg	Neg	Neg	Neg
ESS 50	Morpholine-N-sulphonyl *p*-chloroanilide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 52	Morpholine-N-sulphonyl 2,4-dichloroanilide	Neg	Neg	6000	Neg	Neg	Neg	Neg	Neg
ESS 53	Morpholine-N-sulphonyl α-naphthylamide	Neg	Neg	2400	Neg	Neg	Neg	Neg	Neg
ESS 54	Morpholine-N-sulphonyl β naphthylamide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 51	N,N'-Dimorpholino sulphone	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 67	Morpholine picrate	Neg	Neg	920	1800	Neg	Neg	1900	Neg
Nitro compounds									
UT 10	2,3-Dinitro-2,3-dimethyl-butane	Neg	Neg	84	160	1000	2200	2600	4500
ESS 62	3,5-Dinitro-*o*-cresol	Neg	Neg	18	35	950	1750	130	400
ESS 46	β-Nitrostyrene	630	1550	67	170	1100	2000	1100	3600
ESS 20	*o*-Nitrobiphenyl	Neg	Neg	220	640	Neg	Neg	1750	Neg
ESS 21	*p*-Nitrobiphenyl	Neg	Neg	1060	Neg	Neg	Neg	Neg	Neg
UT 13	2,4-Dinitro-6-cyclohexyl-phenol	2500	Neg	110	195	4700	Neg	1750	4800

TABLE II—*Concluded*

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC$_{50}$) AND LC$_{90}$ FIGURES OF 127 COMPOUNDS
FOR LARVAE OF *Musca, Tribolium,* AND *Ephestia,* AND ADULTS OF *Sitophilus*—Concluded

Expressed in parts per million, by weight

No.	Name of compound	*Musca*		*Sitophilus*		*Tribolium*		*Ephestia*	
		LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$	LC$_{50}$	LC$_{90}$
Miscellaneous nitrogenous compounds									
ESS 25	symm-Diphenylguanidine	Neg	Neg	6500	Neg	Neg	Neg	Neg	Neg
UT 9	Dibutylnitramine	2000	3600	1010	2060	5200	Neg	2700	4200
UT 5	Dicyanodiethylnitramine	Neg	Neg	1400	3000	Neg	Neg	Neg	Neg
UT 11	Di-*n*-butylcyanamide	1550	3200	290	720	3300	Neg	1800	3200
ESS 22	o-Aminobiphenyl	Neg	Neg	750	1200	2700	4400	1100	3600
UT 2	Jablonski compound C$_4$H$_6$N$_4$O	Neg	Neg	7000	Neg	Neg	Neg	Neg	Neg
UT 3	Jablonski compound C$_4$H$_8$N$_4$O$_7$	Neg	Neg	7000	Neg	Neg	Neg	Neg	Neg
Compounds containing C, H, and O only									
ESS 58	Cyclohexylacetic acid	Neg	Neg	600	1000	Neg	Neg	Neg	Neg
ESS 59	Cyclohexylpropionic acid	Neg	Neg	270	450	Neg	Neg	Neg	Neg
ESS 60	Cyclohexylbutyric acid	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 61	Cyclohexylcaproic acid	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 23	o-Hydroxybiphenyl	7000	Neg	860	1320	Neg	Neg	6000	Neg
ESS 3	Coumarin	1400	2900	75	200	4000	5400	1350	4500
Thiocyanates									
ESS 41	Benzyl thiocyanate	230	580	68	140	55	500	330	770
ESS 105	o-Chlorobenzyl thiocyanate	400	830	60	135	1600	3400	320	970
ESS 106	p-Chlorobenzyl thiocyanate	700	1550	94	205	1800	3600	550	2000
ESS 136	2,4-Dichlorobenzyl thiocyanate	1600	3300	640	5000	570	1000	400	1600
ESS 138	3,4-Dichlorobenzyl thiocyanate	Neg	Neg	520	1600	2200	3400	900	2900
ESS 102	α-Thiocyano-1-methyl-naphthalene	Neg	Neg	1210	2000	Neg	Neg	1500	3500
UT 14	Isoborneyl thiocyanoacetate	1700	3500	220	750	—	—	—	—
Miscellaneous sulphur compounds									
ESS 82	β,β'-Dichlorodiethyl sulphoxide	850	2600	2450	Neg	5500	Neg	3400	4800
UT 16	bis(*p*-Chlorophenyl)sulphone	6000	Neg	Neg	Neg	—	—	—	—
McG 5	bis(*p*-Bromophenyl)sulphone	—	—	2100	4500	—	—	Neg	Neg
McG 4	*p*-Bromophenyl sulphonic acid	6300	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 91	Thiourea	3900	Neg	Neg	Neg	4800	7000	400	1300
USDA 1	Thiocoumarin	2300	4300	1050	1310	Neg	Neg	1200	2100
Compounds containing other elements									
UT 17	Phenylboric acid	700	1450	102	305	50	450	150	270
UT 18	o-Nitrophenylboric acid	—	—	1700	Neg	—	—	—	—
ESS 68	Tri-o-cresyl phosphate	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 63	Pyridyl mercuric stearate	3600	Neg	710	1100	Neg	Neg	1500	Neg

Discussion of Results

The results obtained with chlordane and with gammexane are very similar, both compounds showing median lethal concentrations of less than 50 parts per million to all four species. The median lethal concentration of 44 p.p.m. for gammexane against *Musca* larvae by this method is high in comparison with the value of 8 p.p.m. found for benzene hexachloride by McGovran and Piquett (10), who assessed the mortality at the emergence of the adult.

DDT showed a generally high rating, being superior to its analogues. The low toxicity found for methyl-DDT is surprising in view of the excellent results obtained with it against *Pediculus* and *Cimex* by Busvine (4). However, Proverbs and Morrison (11) found that methyl-DDT showed extremely low residual contact toxicity to *Drosophila*. It is also remarkable that methoxy-DDT, recommended for household use because it is alleged to be as toxic as DDT to insects but much less toxic to mammals, showed no toxicity whatever to larvae of *Musca* and *Tribolium*.

The four chlorinated aliphatic hydrocarbons: hexachloropropene, hexachlorobutadiene, and the heptachloropropanes, show a high toxicity by these methods of testing. Although they exhibit no contact toxicity (see Part II of this series of papers) they possess fumigant activity of a high order (unpublished data). Thus their activity is most marked in the case of *Musca*, *Tribolium*, and *Ephestia*, whose larvae live within the closely-packed, non-aerated test medium, whereas in the better ventilated tests with *Sitophilus* adults, these compounds are relatively ineffective.

In addition to phenylboric acid, benzyl thiocyanate would appear to be the most promising new compound revealed by these general tests. It was slightly more toxic than its chloro derivatives and considerably more toxic than its dichloro analogues. Of the five chlorinated analogues of methylbenzene tested, benzotrichloride is most effective, followed by the *o*-chloro and the *p*-chlorobenzyl chlorides.

Of the six nitro compounds tested, four of them were quite strongly insecticidal, while the two nitrobiphenyls were virtually nontoxic. *Sitophilus* in the adult stage was the most susceptible species to these nitro compounds, which include DNOC (dinitro-*o*-cresol), nitrostyrene, dinitrodimethylbutane, and DNOCHP (dinitrocyclohexylphenol). On the other hand, *Musca* larvae showed the least reaction to them, no nitro compounds being included in the 15 most toxic compounds for this species (see Table I), and both DNOC and dinitrodimethylbutane being entirely without effect. The high toxicity of dinitrodimethylbutane is of interest in view of its relationship with 2-nitrobutane, which has shown marked fumigant effect to *Tribolium confusum* (12). The general high rating of β-nitrostyrene is of interest in view of the good results recently obtained by American workers with halogenated derivatives of the closely related nitroethylbenzenes (13).

Of the 15 most toxic compounds to *Musca* larvae, five are cyanides or nitriles. This class of compounds is not represented in the 15 most toxic

compounds for either *Sitophilus* or *Ephestia*. Benzonitrile is in general exceeded in toxicity by phenylacetonitrile (i.e. benzyl cyanide). Of the chlorinated derivatives of benzyl cyanide, the *o*-chloro is more effective against *Musca* and *Ephestia*, while the *p*-chloro is the more toxic against *Tribolium* and *Sitophilus*. The 2,4-dichlorobenzyl cyanide is slightly more effective on the average than the 3,4- derivative. In tests against *Ephestia* and *Tribolium* the chlorinated benzyl cyanides and chlorides were liable to give inconsistent results.

The *p*-nitro derivative of benzyl cyanide (*p*-nitrophenylacetonitrile) was of a lower order of toxicity than the *p*-chloro analogue in these experiments, although it has shown a high order of toxicity to larvae of *Cochliomyia* (3), *Prodenia* (13), and two species of *Culex* (5 and 7). Of the other cyanides tested, phthalonitrile showed a consistently good level of toxicity to all four species, the median lethal concentration obtained for *Musca* being in close agreement with that obtained by McGovran and Piquett (9) by similar methods. Styryl cyanide and the aliphatic nitriles showed moderate to light toxicity.

Thiocoumarin gave results that were consistently inferior to those obtained with coumarin itself. It may be noted that coumarin showed a marked repellent action to *Musca* larvae. The semicarbazones of aromatic ketones, which gave promise in the experiments performed by Swingle, Gahan, and Phillips (14), did not show any outstanding toxicity in these tests, which were made as far as the quantity of available material allowed. As a group the five N-methyl carbamates tested gave poor results. Neither of the two so-called Jablonski compounds, which are cyanamide-formaldehyde condensation products of uncertain composition and doubtful stability, showed any significant toxicity. Of the biphenyls tested, the *o*-amino derivative was found to give the best results; this compound was found to give 50% mortality of *Anopheles* larvae at less than 1 p.p.m. (6). Thiourea was found to be moderately toxic to *Ephestia*, but weakly toxic to the other species, including *Musca*, where the results were inferior to those obtained by McGovran and Piquett (9).

The only morpholine derivatives to show any considerable degree of general toxicity to all four species were the *p*- and *m*-tolylmorpholines. Butyl-morpholine showed an unusually high toxicity to *Ephestia* larvae. Trichloro-acetyl morpholide was markedly toxic to *Tribolium* larvae. Phenyl-morpholine, trichloroacetyl morpholide and morpholine itself gave good results against *Sitophilus* adults.

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References

1. ANONYMOUS. U.S.D.A. adopts Chlordane. Soap Sanit. Chemicals, 23 (4) : 191. 1947.
2. BLISS, C. I. The calculation of the dosage–mortality curve. Ann. Applied Biol. 22 : 134-167. 1935.
3. BUSHLAND, R. C. The toxicity of some organic compounds to young screwworms. J. Econ. Entomol. 33 : 669-676. 1940.
4. BUSVINE, J. R. Insecticidal action of DDT. Nature, 156 : 169-170. 1945.
5. CAMPBELL, F. L., SULLIVAN, W. N., SMITH, L. E., and HALLER, H. L. Insecticidal tests of synthetic organic compounds—chiefly tests of sulfur compounds against culicine mosquito larvae. J. Econ. Entomol. 27 : 1176-1185. 1934.
6. DEONIER, C. C., JONES, H. A., and INCHO, H. H. Organic compounds effective against larvae of *Anopheles quadrimaculatus*—laboratory tests. J. Econ. Entomol. 39 : 459-462. 1946.
7. FINK, D. E., SMITH, L. E., VIVIAN, D. L., and CLABORN, H. V. Toxicity tests with synthetic organic compounds against culicine larvae. U.S. Dept. Agr. Bur. Entomol. Plant Quarantine E-425. 1938.
8. MacNAIR-DORLAND COMPANY. Publishers of Blue Book, Soap and Sanitary Chemicals. The Peet-Grady method. Blue Book: 197-201. 1941.
9. McGOVRAN, E. R. and PIQUETT, P. G. Toxicity of thiourea and phthalonitrile to housefly larvae. J. Econ. Entomol. 36 : 936. 1943.
10. McGOVRAN, E. R. and PIQUETT, P. G. Toxicity of benzene hexachloride to housefly larvae. J. Econ. Entomol. 38 : 719. 1945.
11. PROVERBS, M. D. and MORRISON, F. O. The relative insecticidal activities of DDT and related organic molecules. Can. J. Research, D, 25 : 12-44. 1947.
12. RICHARDSON, H. H., SCHECHTER, M. S., and HALLER, H. L. Toxicity of some nitro paraffins to the confused flour beetle. J. Econ. Entomol. 36 : 111. 1943.
13. SWINGLE, M. C., PHILLIPS, A. M., and GAHAN, J. B. Preliminary tests of synthetic organic compounds as insecticides. I. U.S. Dept. Agr. Bur. Entomol. Plant Quarantine E-621. 1944.
14. SWINGLE, M. C., GAHAN, J. B., and PHILLIPS, A. M. Preliminary tests of synthetic organic compounds as insecticides. II. U.S. Dept. Agr. Bur. Entomol. Plant Quarantine E-634. 1945.

TOXICITY OF SELECTED ORGANIC COMPOUNDS TO INSECTS

PART II. TESTS FOR CONTACT TOXICITY ON NYMPHS OF *BLATELLA* AND *ONCOPELTIS*, AND ADULTS OF *TRIBOLIUM*[1]

By A. W. A. Brown,[2] B. J. Wenner,[2] and F. E. Park[2]

Abstract

The direct contact toxicity of 91 synthetic organic compounds was tested against nymphs of *Blatella germanica* and of *Oncopeltis fasciatus*, and adults of *Tribolium confusum*. The compounds were dissolved in graded concentrations in benzene–kerosene mixture and sprayed on the insects in a spraying tower. Their toxicity was assessed by determination of the median lethal deposits (LD$_{50}$) for each of the three species.

Taking the results with the three species as a whole, the highest contact toxicity was shown by gammexane and chlordane. Dinitro-*o*-cresol and dinitro-cyclohexylphenol were next in order of effectiveness. These were followed by benzyl thiocyanate and its *o*- and *p*-chloro and 2,4-dichloro derivatives. DDT was sixth and methoxy-DDT was 12th on the list of compounds in order of their average effectiveness to the three species. A number of chlorinated aliphatic compounds that were strong fumigants showed no contact toxicity.

Introduction

Of the 127 organic compounds tested for general toxicity to insects and reported in Part I of this series, 91 were sufficiently soluble and in good enough supply to be tested for direct contact toxicity. They were dissolved in a mixture of benzene and kerosene and were applied to three species of insects in a spray tower.

Material

The biological material consisted of fourth and fifth instar nymphs of the German cockroach (*Blatella germanica* L.) reared continuously in the laboratory on compressed meat–vegetable pellets with yeast supplement; last instar nymphs of the large milkweed bug (*Oncopeltis fasciatus* Dall.) continuously reared in the laboratory on seeds of *Asclepias syriaca;* and adults of the confused flour beetle (*Tribolium confusum* Duval) reared continuously in the laboratory on whole wheat flour.

The nymphs of *Blatella* used in the tests were taken after development for 30 to 35 days after hatching at 80° F. and 70% relative humidity. The nymphs of *Oncopeltis* were taken 16 to 19 days after hatching. The adults of *Tribolium* were taken 15 to 25 days after emergence.

The organic compounds to be tested had been synthesized or specially purified for the purpose. The majority (77) were produced by Dr. D. B. W. Robinson and Mr. J. B. Reesor of the Experimental Station, Suffield, Alta., and are designated by the prefix ESS. Fourteen compounds were submitted by Prof. G. F Wright, Department of Chemistry, University of Toronto, and bear the prefix UT.

Methods

The compounds to be tested were dissolved in a mixture of four parts by volume of benzene (reagent grade) and one part of odorless kerosene, the latter oil being added to reduce the volatility of the solvent. Spraying of the oil solutions was carried out in a cylindrical glass spraying tower measuring 29 in. high by 12 in. internal diameter*. The methods followed were in general similar to those described by Tattersfield and Morris (9) and by Busvine (3).

The spray solution was introduced from a 10 cc. burette into the aspirator attachment of a DeVilbiss Special Atomizer No. 631 centrally placed in the lid of the tower. Compressed air was applied to the atomizer at a pressure of 23 cm. Hg measured accurately by an open manometer. The stopcock of the burette was then opened, and the solution passed through a capillary constriction into the atomizer at the rate of 7 cc. per minute.

Four cc. of spray solution were delivered in each case. The room in which spraying was performed was kept at a temperature of 75° to 80° F. and a relative humidity of over 75%, in order to reduce the electrostatic charges generated in the tower. Under these conditions, 4 cc. of spray solution was found to yield an average deposit at the base of the cylinder of approximately 4 cu. mm. of solution per square centimeter. This density of deposit was determined by colorimetric assessment of dyed spray, and prevailed over the central 8 in. of the tower, decreasing toward the walls of the cylinder. Thus where 8% solutions were employed, the density of deposit of the dissolved compound would be 320 gamma per cm.^{2} The average weight of the roaches employed was approximately 50 mgm., and their surface area exposed to the vertical fall of droplets was approximately 0.5 sq. cm. Thus for a deposit of 100 gamma per cm.^{2}, the contact dosage would be 50 gamma per roach, corresponding to 10 mgm. per kgm. body weight. The figures for surface dosage were obtained by printing the outline of the insect on sensitized paper and checked by recovery of dyed spray from a sprayed insect.

The tower was secured against air leaks by a wide band of adhesive tape applied at the junction of the friction lid with the top of the cylinder, and by closing the door by which the test insects were introduced. A half-inch opening in the lid holding a coarse esparto filter served to relieve excess air pressure in the tower. The spray was allowed to settle for one and one-half minute. After each spray test, the burette and atomizer were rinsed with acetone while an evacuating fan drew air towards the base of the tower; after a group of tests the tower was removed and the inner walls were cleaned with acetone.

The size of droplets in the spray was assessed by exposing microscope slides coated with the silicone preparation sold as G. E. Drifilm No. 9987. The diameter of the flattened droplets (lenses) thus obtained were measured under

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* *A convenient supply of glass cylinders is offered by obsolete gasoline pumps, and may be obtained from the Imperial Oil Company.*

the microscope by means of an ocular micrometer. The spreading coefficient, being the ratio between the lens diameter and the diameter of the spherical droplet, was determined according to the method described by May (7). By this method, the droplets were found to range between 5 and 90 μ in diameter, and the median diameter by mass was computed to be 62 μ.

The nymphs of *Blatella* and *Oncopeltis* were immobilized before spraying. After being exposed to a temperature of 34° F. to immobilize them in their rearing containers, they were transferred to Buchner funnels into which carbon dioxide gas was introduced for two minutes. This narcotic treatment kept the insects quiescent for the subsequent 10 to 15 min., during which time they were transferred to crystallizing dishes, placed in a normal posture, exposed in the spray tower, and returned to the observation jars. As may be seen from the control figures reported below, the narcotic treatment was without permanent effect on the insects; nymphs of both species that had been submitted to cold and then to carbon dioxide for 20 min. recovered completely.

The insects were exposed in crystallizing dishes measuring $2\frac{1}{2}$ in. high by $4\frac{3}{4}$ in. in diameter, fitted with a floor of filter paper or paper towelling; 20 nymphs of *Blatella* and of *Oncopeltis*, and 50 adults of *Tribolium* were employed in each test. After being sprayed, the insects were transferred to 2-qt. sealers fitted with screened tops, and were kept at a temperature of 80° F. and 70% relative humidity. The food supplied was meat–vegetable pellets and a vial of water for *Blatella*, milkweed seed and water for *Oncopeltis*, and a 2-in. layer of whole wheat flour for *Tribolium*. Mortality counts of *Blatella* were made 20 hr. later, the period being extended to three days in the case of DDT and its analogues, gammexane, and chlordane. The observation period for *Oncopeltis* was two days, being extended to four days for the above-mentioned slow insecticides. The observation period for *Tribolium* was three days.

The series of sprays performed with each compound was begun by applying 4 cc. of an 8% (wt./vol.) solution, and was continued with successive dilutions of 4, 2, 1, 0.5, 0.125, 0.062, 0 031, and 0.016%, as results warranted proceeding further. Thus the deposits obtained were successively halved with each dilution, whereas the amount of the solvent remained constant. The density of deposit would thus range from 320 gamma per cm.², through 160, 80, 40, etc. down to 0.062 gamma per cm.² Fifteen of the original 127 compounds, not mentioned in this paper, could not be tested owing to their insolubility in liquids suitable for spraying. Twenty-one compounds were not available in sufficient quantity. The boric acid derivatives (UT 17 and 18) were sprayed in a mixture of equal parts of kerosene and 1,4-dioxane. The N-methyl carbamates (ESS 113 to 117) were first dissolved in one part of methyl alcohol and diluted with three parts of the benzene–kerosene mixture.

Of 10 control samples of *Blatella* sprayed with 4 cc. of the solvent alone, the average mortality was 3.5%. Of eight similar control samples of *Oncopeltis*, the average mortality was 0.0%. Of five control samples of *Tribolium*, the mortality was zero.

Results

The percentage mortalities obtained with successive dilutions of the 91 compounds tested against the three species of insects will not be reported here. However, the results obtained with the 20 compounds that showed the greatest contact toxicity to *Blatella* nymphs are shown in Table I.

TABLE I

MORTALITY DATA FOR THE 20 COMPOUNDS THAT WERE MOST TOXIC
BY DIRECT CONTACT TO *Blatella* NYMPHS

Average control mortality: 3.5%

Name of compound (often abbreviated)	% Mortality at the following deposits in gamma/cm.²									
	320	160	80	40	20	10	5	2.5	1.25	0.62
Chlordane	100	100	100	100	100	95	82	75	30	20
Gammexane	100	100	100	100	100	100	70	48	20	—
Dinitro-*o*-cresol	100	100	100	100	88	85	62	10	—	—
Dinitrocyclohexylphenol	100	100	80	70	65	45	15	—	—	—
p-Chlorobenzyl thiocyanate	100	100	85	62	35	10	—	—	—	—
Benzyl thiocyanate	100	98	100	60	15	—	—	—	—	—
Benzyl cyanide	100	100	98	58	3	—	—	—	—	—
DDT	100	90	60	43	37	22	0	—	—	—
o-Chlorobenzyl thiocyanate	100	100	75	75	5	—	—	—	—	—
o-Chlorobenzyl cyanide	90	95	85	15	—	--	—	—	—	—
o-Hydroxybiphenyl	95	98	72	28	10	—	—	—	—	—
p-Chlorobenzyl cyanide	100	90	25	20	—	—	—	—	—	—
p-Chloropropiophenone	98	90	52	12	—	—	—	—	—	—
β-Nitrostyrene	100	70	40	25	16	—	—	—	—	—
Phenylpropionitrile	76	60	52	17	—	—	—	—	—	—
o-Aminobiphenyl	95	80	15	—	—	—	—	—	—	—
Styryl cyanide	100	90	5	—	—	—	—	—	—	—
3,4-Dichlorobenzyl cyanide	62	50	50	10	0	—	—	—	—	—
α-Chloromethylnaphthalene	100	75	15	—	—	—	—	—	—	—
Isoborneyl thiocyanoacetate	80	70	30	5	—	—	—	—	—	—

From the mortality figures so obtained, the median lethal deposit (LD$_{50}$) and the LD$_{90}$ figures were derived for each compound. This was accomplished by plotting the logarithm of the deposit in gamma per cm.² against the probit of the percentage mortality, according to the method of Bliss (1).

The values for LD$_{50}$ and LD$_{90}$ for each of the 91 compounds against the three species employed are tabulated in Table II. The figures are expressed in gamma (μgm.) of the compound per square centimeter of horizontal surface. Where the LD$_{90}$ or LD$_{50}$ was in excess of 350 gamma per cm.² the symbol "Neg" is used to denote nontoxicity at that level. The symbol "–" indicates that the compound, owing to its scarcity or to its insolubility in suitable solvents, was not tested against the species concerned.

TABLE II

APPROXIMATE MEDIAN LETHAL DEPOSITS (LD₅₀) AND LD₉₀ FIGURES OF 92 COMPOUNDS FOR
NYMPHS OF *Blatella* AND *Oncopeltis*, AND ADULTS OF *Tribolium*

Expressed in gamma per sq. cm.

No.	Name of compound	*Blatella*		*Oncopeltis*		*Tribolium*	
		LD₅₀	LD₉₀	LD₅₀	LD₉₀	LD₅₀	LD₉₀
Chlorinated aliphatics							
ESS 107	1,1,1-Trichloroethane	Neg	Neg	—	—	Neg	Neg
ESS 139	2,3-Dichloropropane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 134	2,3-Dichloropropene-1	Neg	Neg	Neg	Neg	Neg	Neg
ESS 83	1,2,3-Trichloropropane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 56	Hexachloropropene	Neg	Neg	Neg	Neg	Neg	Neg
ESS 100	*symm*-Heptachloropropane	400	Neg	Neg	Neg	Neg	Neg
ESS 37	*asymm*-Heptachloropropane	340	Neg	Neg	Neg	Neg	Neg
ESS 81	Octachloropropane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 118	1,4-Dichlorobutane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 57	Hexachlorobutadiene-1,3	Neg	Neg	Neg	Neg	Neg	Neg
Halogenated cyclic compounds							
ESS 90	Gammexane	2 8	5 5	5 2	24	41	130
ESS 111	*o*-Chlorobenzyl chloride	Neg	Neg	Neg	Neg	Neg	Neg
ESS 112	*p*-Chlorobenzyl chloride	320	Neg	Neg	Neg	Neg	Neg
ESS 132	2,4-Dichlorobenzyl chloride	Neg	Neg	320	Neg	Neg	Neg
ESS 133	3,4-Dichlorobenzyl chloride	Neg	Neg	Neg	Neg	Neg	Neg
ESS 109	Benzotrichloride	320	Neg	Neg	Neg	Neg	Neg
ESS 108	Benzotrifluoride	Neg	Neg	Neg	Neg	Neg	Neg
ESS 74	*p*-Chloroacetophenone	210	270	Neg	Neg	Neg	Neg
ESS 75	*p*-Chloropropiophenone	80	185	Neg	Neg	Neg	Neg
ESS 110	*α*-Chloro-1-methylnaphthalene	120	205	Neg	Neg	Neg	Neg
UT 6	5-Chlorofurfural	Neg	Neg	Neg	Neg	Neg	Neg
UT 7	5-Bromofurfural	260	Neg	Neg	Neg	Neg	Neg
UT 1	Cholesteryl chloride	Neg	Neg	Neg	Neg	Neg	Neg
UT 8	Chlordane	1 7	6 5	70	150	50	240
DDT and analogues							
ESS 33	*p,p'*-Dichlorodiphenyltri-chloroethane	40	150	Neg	Neg	27	60
ESS 69	*p,p'*-Diiododiphenyltri-chloroethane	330	Neg	Neg	Neg	Neg	Neg
ESS 70	*p,p'*-Dichlorodiphenyldi-chloroethylene	Neg	Neg	Neg	Neg	Neg	Neg
ESS 72	*p,p'*-Dimethoxydiphenyltri-chloroethane	200	Neg	110	380	Neg	Neg
ESS 71	*p,p'*-Diethoxydiphenyltri-chloroethane	400	Neg	330	Neg	Neg	Neg
UT 16	bis(*p*-Chlorophenyl)sulphone	Neg	Neg	—	—	Neg	Neg
Cyanides and nitriles							
ESS 85	Octanoyl nitrile	Neg	Neg	Neg	Neg	Neg	Neg
ESS 86	Decanoyl nitrile	Neg	Neg	Neg	Neg	Neg	Neg
ESS 87	Tetradecanoyl nitrile	370	Neg	Neg	Neg	Neg	Neg
ESS 88	Octadecanoyl nitrile	340	Neg	Neg	Neg	Neg	Neg

TABLE II—*Continued*

APPROXIMATE MEDIAN LETHAL DEPOSITS (LD$_{50}$) AND LD$_{90}$ FIGURES OF 92 COMPOUNDS FOR NYMPHS OF *Blatella* AND *Oncopeltis*, AND ADULTS OF *Tribolium*—Continued

Expressed in gamma per sq. cm.

No.	Name of compound	*Blatella*		*Oncopeltis*		*Tribolium*	
		LD$_{50}$	LD$_{90}$	LD$_{50}$	LD$_{90}$	LD$_{50}$	LD$_{90}$
Cyanides and nitriles							
ESS 1	Benzonitrile	Neg	Neg	Neg	Neg	Neg	Neg
ESS 2	Benzyl cyanide	35	95	Neg	Neg	Neg	Neg
ESS 103	o-Chlorobenzyl cyanide	55	115	Neg	Neg	Neg	Neg
ESS 104	p-Chlorobenzyl cyanide	80	160	320	Neg	Neg	Neg
ESS 135	2,4-Dichlorobenzyl cyanide	430	Neg	Neg	Neg	Neg	Neg
ESS 137	3,4-Dichlorobenzyl cyanide	100	Neg	Neg	Neg	Neg	Neg
ESS 9	β-Phenylpropionitrile	105	Neg	Neg	Neg	Neg	Neg
UT 4	Styryl cyanide	120	190	Neg	Neg	Neg	Neg
ESS 101	α-Cyano-1-methylnaphthalene	Neg	Neg	Neg	Neg	Neg	Neg
N-Methyl carbamates							
ESS 116	of m-Diethylaminophenol methochloride	120	Neg	—	—	Neg	Neg
ESS 115	of m-Diethylaminophenol methiodide	Neg	Neg	—	—	Neg	Neg
ESS 117	of m-Diethylaminophenol methosulphate	Neg	Neg	—	—	Neg	Neg
ESS 114	of 2-Methyl-5-dimethylamino-phenol methochloride	Neg	Neg	—	—	Neg	Neg
ESS 113	of 2-Methyl-5-dimethylamino-phenol methiodide	Neg	Neg	—	—	Neg	Neg
Morpholine compounds							
ESS 10	Morpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 12	N-Ethylmorpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 14	N-Butylmorpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 73	N-(Trichloroacetyl) morpholide	380	Neg	Neg	Neg	Neg	Neg
ESS 15	N-Phenylmorpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 89	N-(p-Chlorophenyl) morpholine	380	Neg	Neg	Neg	Neg	Neg
ESS 17	N-Benzoyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 29	N-(p-Tolyl)morpholine	165	Neg	Neg	Neg	Neg	Neg
ESS 43	N-(p-Chlorobenzoyl) morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 45	N-(o-Chlorobenzoyl) morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 26	Benzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 27	p-Bromobenzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 28	p-Toluenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
Nitro compounds							
UT 10	2,3-Dinitro-2,3-dimethylbutane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 62	3,5-Dinitro-o-cresol	5.5	15	6	11	140	270
ESS 46	β-Nitrostyrene	90	220	90	180	Neg	Neg
ESS 20	o-Nitrobiphenyl	250	Neg	Neg	Neg	Neg	Neg
ESS 21	p-Nitrobiphenyl	360	Neg	Neg	Neg	Neg	Neg
UT 13	2,4-Dinitro-6-cyclohexylphenol	16	75	6	13	300	Neg

TABLE II—*Concluded*

APPROXIMATE MEDIAN LETHAL DEPOSITS (LD$_{50}$) AND LD$_{90}$ FIGURES OF 92 COMPOUNDS FOR
NYMPHS OF *Blatella* AND *Oncopeltis*, AND ADULTS OF *Tribolium*—Continued

Expressed in gamma per sq. cm.

No.	Name of compound	Blatella		Oncopeltis		Tribolium	
		LD$_{50}$	LD$_{90}$	LD$_{50}$	LD$_{90}$	LD$_{50}$	LD$_{90}$
Miscellaneous nitrogenous compounds							
UT 9	Dibutylnitramine	180	Neg	Neg	Neg	Neg	Neg
UT 5	Dicyanodiethylnitramine	Neg	Neg	Neg	Neg	Neg	Neg
UT 11	Di-*n*-butylcyanamide	155	300	Neg	Neg	Neg	Neg
UT 12	*p,p'*-Dichlorobenzophenone oxime	Neg	Neg	Neg	Neg	Neg	Neg
ESS 22	*o*-Aminobiphenyl	115	235	Neg	Neg	Neg	Neg
ESS 91	Thiourea	370	Neg	Neg	Neg	Neg	Neg
Compounds containing C, H, and O only							
ESS 58	Cyclohexylacetic acid	280	Neg	380	Neg	Neg	Neg
ESS 59	Cyclohexylpropionic acid	180	Neg	Neg	Neg	Neg	Neg
ESS 60	Cyclohexylbutyric acid	160	Neg	Neg	Neg	Neg	Neg
ESS 61	Cyclohexylcaproic acid	Neg	Neg	Neg	Neg	Neg	Neg
ESS 23	*o*-Hydroxybiphenyl	60	155	Neg	Neg	Neg	Neg
ESS 3	Coumarin	260	Neg	Neg	Neg	Neg	Neg
Thiocyanates							
ESS 41	Benzyl thiocyanate	35	70	80	170	Neg	Neg
ESS 105	*o*-Chlorobenzyl thiocyanate	45	90	55	130	Neg	Neg
ESS 106	*p*-Chlorobenzyl thiocyanate	30	80	33	55	Neg	Neg
ESS 136	2,4-Dichlorobenzyl thiocyanate	380	Neg	50	90	Neg	Neg
ESS 138	3,4-Dichlorobenzyl thiocyanate	Neg	Neg	70	105	Neg	Neg
ESS 102	*α*-Thiocyano-1-methylnaphthalene	Neg	Neg	Neg	Neg	Neg	Neg
UT 14	Isoborneyl thiocyanoacetate (Fraction 2)	130	Neg	90	120	Neg	Neg
UT 15	Isoborneyl thiocyanoacetate (Fraction 5)	Neg	Neg	—	—	Neg	Neg
Compounds containing other elements							
UT 17	Phenylboric acid	Neg	Neg	—	—	Neg	Neg
UT 18	*o*-Nitrophenylboric acid	Neg	Neg	—	—	Neg	Neg
ESS 68	Tri-*o*-cresyl phosphate	Neg	Neg	—	—	Neg	Neg

Discussion of Results

The resistance of adults of *Tribolium confusum* to contact sprays of the organic compounds tested is remarkable, since the great majority of them caused no mortality whatsoever. Only five compounds, namely DDT, gammexane, chlordane, DNOC, and DNOCHP, were powerful enough to induce a significant degree of mortality. All of these are proven insecticides, and moreover are toxic to *Tribolium* at quite low dosages.

Both gammexane and chlordane showed a consistently high level of toxicity to the three species tested. The toxicity of gammexane to *Blatella germanica* has already been reported in general terms by Slade (8). Good results with gammexane applied as a contact insecticide have recently been communicated by Bottger and Levin (2) for agricultural insects. The superiority of chlordane as a contact poison for the cockroach *Periplaneta americana* has been reported by Kearns, Ingle, and Metcalf (6), who found this material to be superior to DDT against many species of insects.

Although DDT was found to be the most toxic of the compounds tested against *Tribolium* adults, it proved to be one of the less toxic materials for *Blatella* nymphs. Moreover it exhibited no toxicity whatever* to *Oncopeltis* nymphs. The nontoxicity of DDT to certain insect species has been noted by many workers. None of the analogues of DDT tested showed any appreciable toxicity to either *Blatella* or *Tribolium*, although methoxy-DDT was moderately toxic, and ethoxy-DDT slightly toxic, to *Oncopeltis*.

These results show that DNOC (3,5-dinitro-*o*-cresol) is an effective contact insecticide for the German cockroach, as it is to grasshoppers and locusts, related orthopterans. Moreover it shows considerable contact toxicity to nymphs of *Oncopeltis*, representing the order Hemiptera. It is followed in toxicity by 2,4-dinitro-6-cyclohexylphenol, a nitro compound cited as being generally superior to DNOC by Kagy (5). β-Nitrostyrene was a third nitro compound to show high contact toxicity.

The most promising of the compounds newly synthesized for this project were benzyl thiocyanate and its *o*- and *p*-chloro derivatives. These three thiocyanates showed greater contact toxicity than Lethane 60 or Lethane 384, which were tested for comparison. They were also superior to a sample of isobornyl thiocyanoacetate obtained from the insecticidal material Thanite. Benzyl thiocyanate has been found by Hartzell and Wilcoxon (4) to be an effective contact insecticide for *Aphis rumicis*, although it formed poor emulsions. None of the thiocyanates showed toxicity to *Tribolium* adults.

A considerable number of the cyanides and nitriles tested showed moderate contact toxicity to *Blatella* nymphs. Benzyl cyanide (phenylacetonitrile), phenylpropionitrile, styryl cyanide, the *o*- and *p*-chlorobenzyl cyanides and 3,4-dichlorobenzyl cyanide, all showed median lethal concentrations of between 120 and 35 gamma per cm.[2] However, none had any effect on *Tribolium* adults, and only *p*-chlorobenzyl cyanide showed slight toxicity to *Oncopeltis* nymphs.

It is of interest to note that the α-chlorotoluene analogues, namely the chlorobenzyl and dichlorobenzyl chlorides as well as benzotrichloride, showed a negligible degree of contact toxicity to any of the three species tested. Moreover the group of halogenated aliphatic compounds, many of which have shown a general and fumigant toxicity of a high order, exhibited little or no effect as contact sprays.

However, at the end of the four-day observation period, the insects did exhibit varying degrees of DDT jitters.

Acknowledgments

References

1. BLISS, C. I. The calculation of the dosage–mortality curve. Ann. Applied Biol. 22 : 134-167. 1935.
2. BOTTGER, G. T. and LEVIN, C. Comparative toxicity to insects of benzene hexachloride and DDT. J. Econ. Entomol. 39 : 539-541. 1946.
3. BUSVINE, J. R. Pioneer work on protection against body lice by the use of lasting insecticides. Bull. Entomol. Research, 36 : 23-32. 1945.
4. HARTZELL, A. and WILCOXON, F. Organic thiocyanogen compounds as insecticides. Contrib. Boyce Thompson Inst. 6 : 269-277. 1934.
5. KAGY, J. F. The relative toxicity of some 2,4-dinitro-6-R-phenols. J. Econ. Entomol. 34 : 660-669. 1941.
6. KEARNS, C. W., INGLE, L., and METCALF, R. L. A new chlorinated hydrocarbon insecticide. J. Econ. Entomol. 38 : 661-668. 1945.
7. MAY, K. R. The cascade impactor: an instrument for sampling coarse aerosols. J. Sci. Instruments, 22 : 187-195. 1945.
8. SLADE, R. E. The γ-isomer of hexachlorocyclohexane (gammexane). An insecticide with outstanding properties. Chemistry & Industry, 40 : 314-319. 1945.
9. TATTERSFIELD, F. and MORRIS, H. M. An apparatus for testing the toxic values of contact insecticides under controlled conditions. Bull. Entomol. Research, 14 : 223-233. 1924.

ON A *PHYSALOPTERA* LARVA FROM AN INSECT[1]

BY M. A. BASIR[2]

Abstract

A *Physaloptera* larva is described from the body cavity of the earwig, *Labidura reparia* (Pallas).

About 50 earwigs were examined for nematode infection. No adult nematodes were obtained but two small larval specimens were found free and unencysted in the body cavity of one of them. These belong to the genus *Physaloptera* and are described below. Both the specimens are of approximately the same size.

Physaloptera sp.

$$\text{(Larva)} \quad \frac{1.43}{4.05} - \frac{?}{?} \quad \frac{48.8}{6.48} \quad \frac{J}{5.94} \quad \frac{94 \ 29}{3.33} \quad 2.1 \text{ mm.}$$

The body length is 2.1 mm. The cuticle is striated only in the anterior one-fifth of the body, up to a distance of about 400μ from the anterior end, where striation suddenly ends. The larva is broadest at the base of the oesophagus, being about 130μ wide; from here it gradually tapers towards the tail end. In the region of the oesophagus the body is more or less cylindrical. Narrow lateral alae are present throughout the length of the body.

The head is triangular in outline and is 30μ high. It is formed of two massive pseudolabia of equal size. Each pseudolabium bears a pair of papillae, one of which is dorsolateral and the other ventrolateral in position. The papillae are typically physalopteroid in form and position. Amphids or lateral organs appear as minute openings situated laterally on the pseudolabia. The mouth opening is flattened and is elongated dorsoventrally. It appears like a long narrow slit. Each pseudolabium bears four teeth on its antero-lateral aspects. One tooth is comparatively large and is borne almost at the tip of the pseudolabium on the externolateral side. This can also be seen in lateral view. The other three teeth are placed in a row internal to the large tooth, on the anterior internolateral sides of each lip. These are not sharply

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pointed at this stage of the larva. They show a tendency to appear rather like three small lobes of the pseudolabium. This character appears to be important from the point of view of the affinities of the genus *Physaloptera* and may indicate the evolution of the three internolateral physalopteran
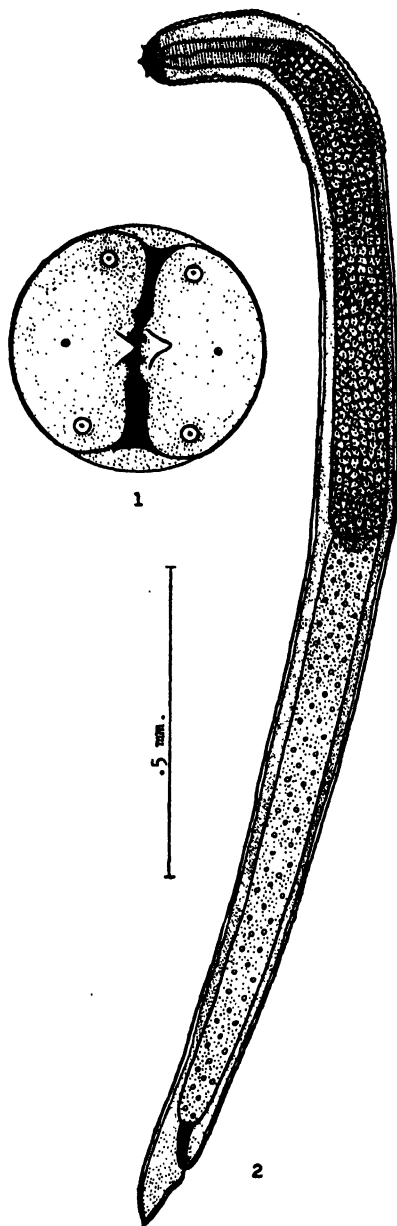
FIG. 1. *En face view.*
FIG. 2. *Lateral view.*

teeth from the trilobed pseudolabia of the other spiruroids, particularly the Gnathostomatidae (2, 3).

The buccal cavity is in the form of a vestibule enclosed by the pseudolabia and is not clearly seen. It is about 30μ deep. The oesophagus extends to the base of the pseudolabia. It is 995μ long and is distinctly divided into two portions. The short anterior cylindrical part is 185μ long by 45μ wide. In the larva under examination, it does not clearly show the development of the muscles, but there can be no doubt that it corresponds to the typical anterior muscular oesophagus of the genus. The posterior part of the oesophagus is very long and broad, being 810μ in length and 90μ in width. The granules in this portion of the oesophagus are heavy and dense, rather coarse and having an alveolar appearance. A lumen can be seen only in the anterior part of the oesophagus. At the posterior end it communicates with the intestine through a pair of valves that appear to be cuticularized.

The intestine originates as a thinner tube than the oesophagus, being only 70μ broad near the start. It communicates posteriorly with a short and narrow rectum, which is 80μ long. No rectal glands are seen. The anus is situated at a distance of 110μ from the posterior end of the body. The tail is conical in form. No papillae or spines are seen on it.

The nerve ring could not be observed. The excretory pore is situated at a distance of about 220μ from the anterior end of the body.

Host: *Labidura reparia* (Pallas) (Order: Dermaptera).

Habitat: Body cavity (found free in the body cavity).

Locality: Aligarh (North India).

Discussion

Although the genus *Physaloptera* contains over 50 species from a large variety of hosts including amphibians, reptiles, birds, and mammals, the complete life history of none of them has been worked out up to this time. Seurat (6) gave the description of several third and fourth stage *Physaloptera* larvae from the definitive vertebrate hosts. These descriptions no doubt help to indicate the form of the infective larvae one would expect to find in the intermediate host. Mirza (5) described *Physaloptera* larvae that he found encysted in the body cavity of the Indian squirrel, but whether the latter serves as a proper intermediate host or was an erratic host is not known. Alicata (1) for the first time succeeded in developing the eggs of *Physaloptera* in an intermediate host up to the third stage larvae. He fed the eggs of *Physaloptera turgida* to the cockroach *Blatella germanica*. The worms were taken from an opossum. He states that the first and second stage larvae were found free in the body cavity of the cockroach but the third stage larvae, which took about four weeks to grow, were found encysted in the tissue surrounding the body cavity and were coiled loosely within the cyst. About seven weeks after the initial infection these larvae were found encysted in the body cavity. Alicata states further that some of these "encysted third stage larvae were found to be enclosed in a thin, brownish chitinous-like substance,

probably representing a deposit derived from the tissue of the cockroach. This would probably represent a defense reaction to a foreign invader. . . These deposits appear first usually at the anterior and posterior extremities of the larva, and gradually spread until the larva is enclosed within a tube formed by these deposits. Eventually the larva is killed and becomes completely chitinized." This indicates that *Blatella germanica* can serve as an intermediate host for *P. turgida* but is probably not the natural intermediate host. The infective larvae were fed to a dog, a cat, a rabbit, a guinea pig, a rat, and a chick; these animals were killed and examined after a month but no adults were found in them. Hobmaier (4) developed the eggs of *Physaloptera maxillaris* Molin, also in *Blatella germanica*, up to the infective larval stage. He also states that one or more of these larvae may be found encysted in the body cavity of the cockroach and that "some of these cysts may show a golden brownish colour similar to that of the cuticle of the cockroach, with or without destruction of the enclosed larvae." On feeding the infected cockroaches to cats, dogs, and guinea pigs he did not get any adults.

The larvae described in this paper were found free in the body cavity of the insect. This appears to be the first recorded natural infection of a *Physaloptera* larva in an intermediate host.

References

1. ALICATA, J. E. Larval development of the spirurid nematode, *Physaloptera turgida*, in the cockroach *Blatella germanica*. Papers on Helminthology, Jub. Skrjabin, All-Union Lenin Acad. Agr. Sci., Moscow, 11-14. 1937.
2. CHITWOOD, B. G. and CHITWOOD, M. B. An introduction to nematology. Sec. 1, Part 2. Babylon, N.Y. 1938.
3. CHITWOOD, B. G. and WEHR, E. E. The value of cephalic structures as characters in nematode classification, with special reference to the superfamily Spiruroidea. Z. Parasitenk. 7 : 273-335. 1934.
4. HOBMAIER, M. Extramammalian phase of *Physaloptera maxillaris* Molin, 1860 (Nematoda). J. Parasitol. 27 : 233-235. 1941.
5. MIRZA, M. B. *Sciurus palmarum* als ein interessanter wirt von *Physaloptera* sp. Z. Parasitenk. 6 : 638-641. 1934.
6. SEURAT, L. G. Contribution a l'étude des formes Larvaires des nématodes parasites Hétéroxènes. Bull. Sci. France Belg. 49 : 297-377. 1916.

CAMERONIA BIOVATA GEN. ET SP. NOV. (THELASTOMATIDAE), A NEW NEMATODE PARASITE OF THE MOLE CRICKET, *GRYLLOTALPA AFRICANA* BEAUV.[1]

BY M. A. BASIR[2]

Abstract

Cameronia biovata gen. et sp. nov. is described from *Gryllotalpa africana* Beauv. This new genus can be differentiated from the genus *Binema* Trav., 1925, the only genus in the subfamily Thelastomatinae to which it shows some resemblance, by the position of the vulva, which is much posterior in the former, and by its characteristic eggs, which are without polar filaments and are laid in pairs, both the eggs losing their separate identity by fusing with each other along their flattened surfaces, slightly asymmetrically and in a constant and typical pattern.

In his two previous papers (1, 2) the writer has described some new nematodes found as parasites of *Gryllotalpa*. During further study of the same group of insects a new form was met with, which also belongs to the subfamily Thelastomatinae of the family Thelastomatidae. In the opinion of the writer it represents a new genus and a new species of the subfamily Thelastomatinae. The name *Cameronia biovata** is proposed for it.

Description

Genus *Cameronia* gen. nov.

Generic diagnosis: Thelastomatinae.

Male unknown.

Female with mouth opening circular, surrounded by a circumoral elevation and eight labiopapillae. Buccal cavity short and cylindrical, partly surrounded by the oesophagus and containing one dorsal and two subventral cuticular elevations. Oesophagus consisting of a corpus, an isthmus, and a posterior valvular bulb. Excretory pore posterior to base of oesophagus. Tail conical. Vulva in the posterior third of the body. Ovaries two; vagina long and muscular, directed anteriorly. Divergent uteri meet near the middle of body to form a single common uterus, which runs backwards to join the vagina. Eggs elliptical, flattened on one side and fused in pairs along their flattened surfaces, slightly asymmetrically, but in a constant and regular pattern; externally covered by a common cuticular covering; laid in morula stage; polar filaments absent.

Type species: Cameronia biovata *sp. nov.*

Specific diagnosis: Cameronia.

Male unknown.

Female 2.35 to 2.50 mm. long by 400μ in maximum width. Cuticle striated throughout the body except the tail. First annule 15μ wide, annules in the cervical region about 7μ apart; towards the middle of the body they increase in width and reach a maximum width of 15μ. Mouth opening circular, surrounded by a circumoral elevation and eight submedian labiopapillae. Buccal cavity short and cylindrical, partly surrounded by the oesophagus, 10μ deep by 10μ wide, and containing one dorsal and two subventral cuticular
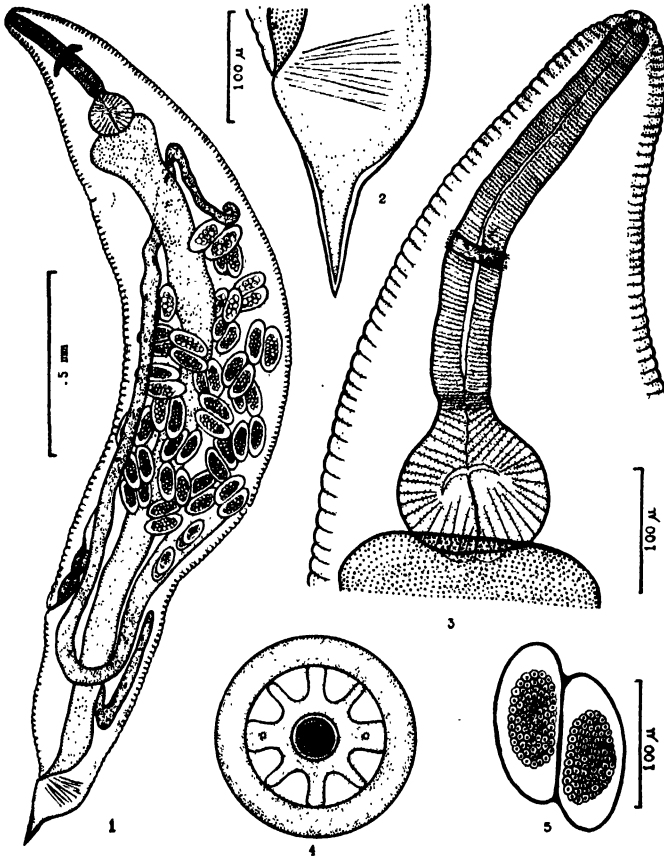
FIG. 1. *Female, entire, lateral view.* FIG. 2. *Female, tail.* FIG. 3. *Female, oesophageal region.* FIG. 4. *Female, en face view.* FIG. 5. *Eggs.*

elevations. Oesophagus 440 to 465μ long, consisting of a corpus 317 to 335μ long by 45μ wide, an isthmus 20μ long by 40μ wide, and a posterior valvular bulb 125 to 150μ long by 230μ wide. Nerve ring 200μ from the anterior end of body. Excretory pore posterior to base of oesophagus, 500μ from the anterior end of body. Intestine enlarged anteriorly to form a cardia. Anus

180 to 190μ from the posterior end of body. Tail conical. Vulva 1.7 mm. from the anterior end of body, about 72% of the body length from the anterior end. Ovaries two, vagina long and muscular, meeting a common uterus that runs anteriorly up to the middle of body, then branching into two divergent uteri. Eggs elliptical, flattened on one side and fused in pairs along their flattened surfaces with a slight asymmetry, one-fifth of the length of each egg projecting free on opposite sides. Each pair secondarily covered over by a common cuticular layer. Eggs measure 130μ in length by 50μ in width, and are laid in the morula stage.

Host: *Gryllotalpa africana* Beauv.

Location: Intestine (rectum).

Type locality: Aligarh (North India).

<h2 style="text-align:center">Discussion</h2>

The only genus in the subfamily Thelastomatinae that the genus *Cameronia* resembles is *Binema* Travassos, 1925 (3, p. 12, Fig. 11 (*h*); 5, Figs. 1-6). However, it differs from it in the following points. In the genus *Binema* the vulva is described by Travassos as median in position, while in *Cameronia* it lies in the posterior third of the body. In the former the eggs bear tufts of polar filaments and are laid in capsules, each capsule generally enclosing two eggs or, rarely, three or four. According to Christie (4, p. 250, Fig. 166 (*g*)) the capsules are "of loose texture formed, apparently, by the entangling and anastomosing of polar filaments," while Valkanov (6) is of the opinion that they are formed as a secondary secretion of the oviduct. In the genus *Cameronia* the eggs have a totally different pattern. They are much bigger in size, being more than double the length of those of *Binema*, and are flattened on one side. They bear no polar filaments, and no capsule of the type described for the genus *Binema* is formed here. The eggs are joined together in pairs by their flattened surfaces, not in complete apposition but with one-fifth of the length of each egg projecting free on opposite sides (Fig. 5). The joining surfaces apparently fuse into each other and the pair is secondarily covered by a layer of cuticular secretion. The eggs thus lose their separate identity and appear as fused in pairs, while in *Binema*, although enclosed in capsules, they remain separate and do not actually fuse with each other.

<h2 style="text-align:center">References</h2>

1. BASIR, M. A. Nematodes parasitic in *Gryllotalpa*. Records Indian Museum, 44 : 95-106. 1942.
2. BASIR, M. A. *Chitwoodiella ovofilamenta*, gen. et sp. nov., a nematode parasite of *Gryllotalpa*. Can. J. Research, D, 26 : 4-7. 1948.
3. CHITWOOD, B. G. and CHITWOOD, M. B. An introduction to nematology. Sec. 1, Part 1. Washington, D.C. 1937.
4. CHRISTIE, J. R. An introduction to nematology. Sec. 2, Part 2. Babylon, New York. (Undated).
5. TRAVASSOS, L. Quelques nématodes du *Gryllotalpa*. Compt. rend. soc. biol. 93 : 140-141. 1925.
6. VALKANOV, A. Über die anatomie und cytologie der nematode *Binema binema* Travassos. Trav. soc. Bulgare sci. nat. 17 : 153-167. 1936. (In Bulgarian with German summary.)

PARASITES OF FRESHWATER FISH

IV. INTERNAL HELMINTHS PARASITIC IN SPECKLED TROUT (*SALVELINUS FONTINALIS* (MITCHILL)) IN RIVERS AND LAKES OF THE LAURENTIDE PARK, QUEBEC, CANADA[1]

By L. P. E. Choquette[2]

Abstract

The distribution and incidence of the following species of helminths from speckled trout in lakes and rivers of the Laurentide Park are recorded: *Crepidostomum cooperi*, *C. farionis*, *Phyllodistomum lachancei*, *Eubothrium salvelini*, diphyllobothriid larvae, *Ligula intestinalis*, *Proteocephalus parallacticus*, proteocephalid larva sp. inq., *Rhabdochona laurentiana*, *Metabronema canadense*, *Agamospirura* sp. inq., *Echinorhynchus lateralis*. The sampling includes 42 lakes and streams from seven different drainage systems. The most commonly found species are: *Metabronema canadense*, *Crepidostomum cooperi*, *Eubothrium salvelini*, and *Echinorhynchus lateralis;* these occur in all seven drainage systems. The other helminths vary in their distribution and incidence.

Helminthic infections of freshwater fish in the Province of Quebec have been dealt with in previous papers by Lyster (8) and Miller (12).

The present study of the distribution and incidence of helminthic infection of speckled trout (*Salvelinus fontinalis*) in the Laurentide Park forms a part of the larger study, initiated in 1943, of the biology and parasitology of this fish in the Laurentide area. The work is being carried out in collaboration with the Quebec Department of Fish and Game.

The material under study came mainly from fish collected by field parties from this Institute during the summers of 1945 and 1946; a few specimens were collected during the summer of 1947.

Usually the fish were eviscerated shortly after capture, and the visceral contents examined with the aid of a dissecting microscope; if helminths were present the material was preserved in 5% formalin and sent to the laboratory for more detailed examination. A total of 210 whole fish, preserved in formalin, also was sent to the laboratory for individual examination. Only the findings from these preserved whole fish were used in computing the incidence of infection as given below. Records of some helminthological material collected by the personnel of the Office of Biology, Province of Quebec, during the summers of 1938 and 1939, and sent to this Institute for identification, are also included in this study.

In only one species (*Phyllodistomum lachancei* Choquette) were observations made on living specimens. Alum carmine and Delafield's haematoxylin were

used in the staining of trematodes, cestodes, and Acanthocephala; nematodes were studied after being cleared in lactophenol.

Forty-two lakes and rivers from seven different drainage systems were included in the survey. The lakes and rivers are distributed in the various drainage systems as follows: Chicoutimi, 20; Montmorency, 3; Métabet-chouan, 2; Belle-Rivière, 2; Ste-Anne-du-Nord, 3; Malbaie, 2; Jacques-Cartier, 10. As the sampling was done only in summer, no concept of seasonal distribution can be obtained as a result of this investigation. The accom-panying map (Fig. 1) shows the configuration of the drainage systems.
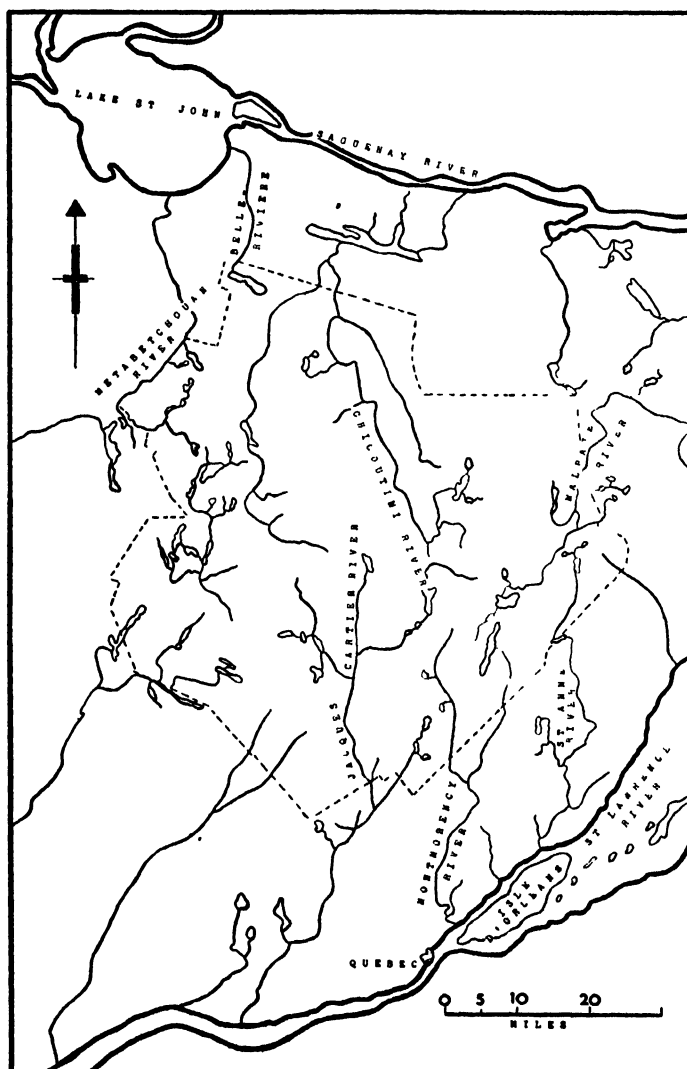
FIG. 1.   *Configuration of the drainage system.*

Distribution and Incidence of Infection

Table I lists the drainage systems in which the various species of parasites were found, together with the number of lakes and streams in each system in which infections were recorded. The detailed records on which this table is based are on file at the Institute of Parasitology.

A brief discussion of the different parasites follows.

TABLE I

Species found and number of lakes or rivers in which it was recorded	Drainage systems*						
	1	2	3	4	5	6	7
	Number of lakes and rivers examined						
	20	10	3	2	2	2	3
Crepidostomum cooperi	16	6	3	1	2	2	2
C. farionis	0	2	1	0	0	0	0
Phyllodistomum lachancei	0	2	1	0	0	1	1
Eubothrium salvelini	12	7	1	2	2	2	1
Ligula intestinalis	1	0	0	0	0	0	0
Proteocephalidae sp.	3	5	1	0	0	0	0
Diphyllobothriid larvae	7	4	1	0	1	0	1
Metabronema canadense	20	9	3	2	2	2	2
Rhabdochona laurentiana	3	1	0	0	0	0	0
Agamospirura sp. inq.	3	2	0	0	0	0	1
Echinorhynchus lateralis	13	6	1	1	2	2	2

* Chicoutimi River = 1   Belle-Rivière = 5
   Jacques-Cartier = 2   Malbaie = 6
   Montmorency = 3   Ste-Anne-du-Nord = 7
   Métabetchouan = 4

Trematodes

Two species of the genus *Crepidostomum* (Allocreadiidae) and one of the genus *Phyllodistomum* (Gorgoderidae) were recorded. "Black spot" was found to be widely but erratically distributed throughout the area studied. A separate account of the distribution of this condition is in preparation. Lyster (9) in 1940, working in another area of the Laurentian mountains in Quebec, showed that it is caused by the trematode parasite *Apophallus brevis*.

Genus *Crepidostomum* Braun, 1900

Of the two species of *Crepidostomum* recorded from trout, *C. cooperi* is the more prevalent in this area. It was found in fish from all seven drainage systems. It was found in 107 of the 210 fish examined individually. While it usually lives in the intestine and the pyloric caeca, in several instances it was found in the gall bladder and stomach. *C. farionis* (Fig. 2) does not seem to have as wide a distribution as *C. cooperi*. It was recorded from fish of three lakes: Lac Horatio Walker and Lac Sept-Isles of the Jacques-Cartier river system, and in Lac des Roches of the Montmorency river system.

Species of *Crepidostomum* have been reported by other workers from Quebec and Ontario. The earliest report is that of Stafford (16) whose findings have been commented upon by Nicoll (Hunninen and Hunter (6)),
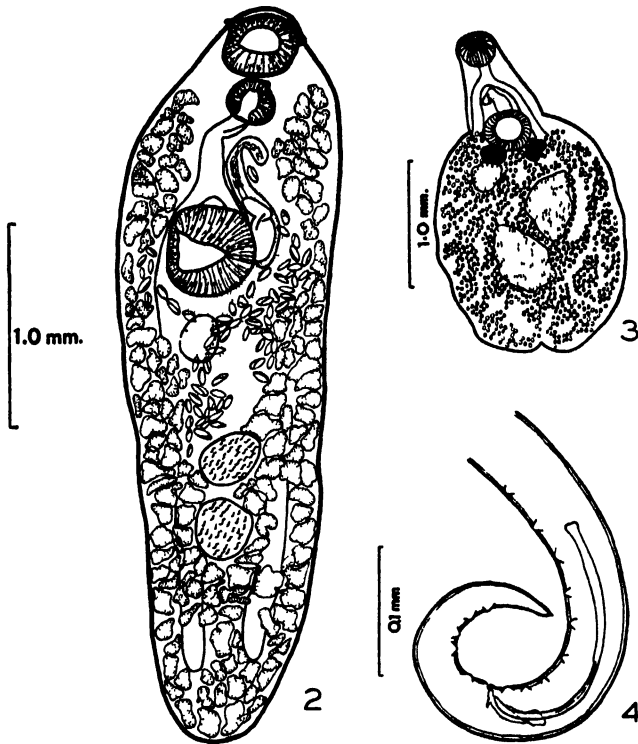
FIG 2   *Crepidostomum farionis, ventral view.*
FIG. 3.   *Phyllodistomum lachancei, ventral view.*
FIG 4.   *Rhabdochona laurentiana, posterior extremity of male*

Hopkins (5), and Miller (13).   Richardson (14), in 1936, recorded *C. cooperi* (under the name of *C. fausti*) from Lake Edward, Champlain County, Que. Lyster (8) found *C. cooperi* in speckled trout from Lake Commandant.   The only other records of *C. farionis* in this country are those of Bangham and Venard (1), and MacLulich (11) who recorded it from speckled trout in the Algonquin Provincial Park in Ontario.

Genus *Phyllodistomum* Braun, 1899 (Fig. 3)

Only one species of *Phyllodistomum* was found in trout.   The trematode occurred in the ureters of the host, always in small numbers, not more than eight individuals being present in any one fish.   This trematode proved to be a previously undescribed species, and was described and named *Phyllodistomum lachancei* by the author (4).   Sections of the ureters and kidneys of the parasitized fish show that the pathological changes caused by the parasite consist of a marked enlargement of the ureteral lumen and a flattening of the

lining columnar epithelial cells.   No evidence of damage to the parenchymal tissue was found.   This parasite was found in two lakes of the Jacques-Cartier river drainage (Lac à Regis and Lac Horatio Walker), in Lac Carré of the Malbaie system, in Lac des Roches of the Montmorency system, and in Lake Turgeon of the Ste-Anne-du-Nord drainage.

Cestodes

Eubothrium salvelini (Schrank, 1790)

The systematic position of species of this genus in Canadian fish has been discussed by Wardle (19) and Kuitunen-Ekbaum (7).   *E. salvelini* has been recorded from trout in other parts of the province by Richardson (14), and Lyster (8).   It was also recorded by MacLulich (11) and Bangham and Venard (1) from Ontario.

Mature and immature forms were found in 60 of the 210 fish examined.   In most cases the worms were located in the pyloric caeca and to a lesser extent in the intestine; a few specimens were also found in the stomach.   This, cestode was prevalent in fish from all seven drainage systems.

Diphyllobothriid larvae

Although these were the larval cestodes most commonly encountered, only 11 fish out of the 210 examined, were infected.   They were found encysted on the outer surface of the stomach, intestine, and kidney, but in small numbers only in any one host.   The infection was found in five of the drainage systems, namely: Chicoutimi, Jacques-Cartier, Montmorency, Ste-Anne-du-Nord, and Malbaie.   MacLulich (11), in a similar survey of the lakes in Algonquin Park, does not record the presence of these forms in speckled trout.

Ligula intestinalis (Linn. 1758)

This species was found only once, in the body cavity of a trout from the River Chicoutimi.   This finding constitutes the only record of the parasite in the speckled trout in Canada.

Proteocephalidae (syn. Ichthyotaeniidae)

Larval stages and immature adults of proteocephalid cestodes were found in the intestine of a small number of fish in a few lakes from the Chicoutimi, Jacques-Cartier, and Montmorency drainage systems.   The immature adults were, on the basis of the characters exhibited by the vagina in its relationship to the cirrus pouch, identified as *Proteocephalus parallacticus* as described by MacLulich (10).   However, in other lakes in these three drainage systems larval stages of members of this family were found but these could not be identified as to species.   They occurred only in very small numbers.   As pointed out by Van Cleave and Mueller (17), the presence of such larvae is of common occurrence in fish that cannot bring them to maturity.   Lyster (8) records such larvae in speckled trout from Lake Commandant.

Nematodes

Nematode infections of trout in this area are represented by one species of the Thelaziidae and one adult and one larval species of Spiruridae. This type of infection resembles closely that encountered by Lyster (8) in other parts of the province. It is quite different from those encountered by Bangham and Venard (1) and MacLulich (11) in the Algonquin Park, and none of the species recorded here was found by Richardson (14) in his study of the parasites of speckled trout.

Rhabdochona laurentiana Lyster, 1940 (Fig. 4)

This species was seen in a few fish from lakes of the Chicoutimi and Jacques-Cartier drainage systems, but was present in very small numbers in any one fish. Lyster (8) described it originally from Lake Commandant, Quebec.

Metabronema canadense Skinker, 1931

Skinker (15) described this species from speckled trout taken in the River Matamek, Quebec Province. This nematode was the helminth most commonly encountered during this survey and was collected from 139 of the 210 fish examined. The parasite was found usually in the stomach, less frequently in the pyloric caeca and intestine.

Lyster (8) recorded *Metabronema* (= *Cystidicola*, *Cystidicoloides*) *harwoodi* from the same host in another part of the province. This species was originally described by Chandler (3) from speckled trout. However, re-examination of Lyster's type material showed it to be identical with *M. canadense*.
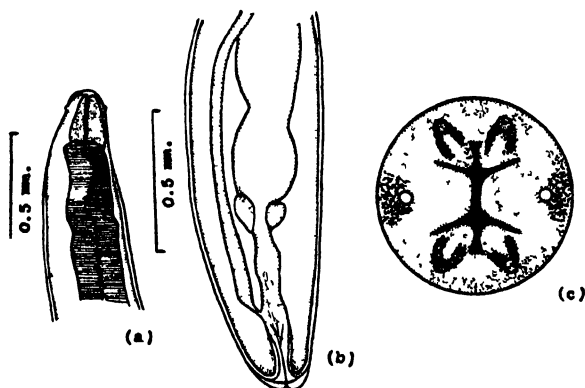
FIG. 5. *Agamospirura sp. inq.*
(a) *Lateral view of anterior extremity.*
(b) *Posterior extremity*
(c) *Sketch of cephalic pattern.*

Agamospirura sp. (Fig. 5, a, b, c)

Eleven immature spirurids were found in fish taken during the summer of 1945 and 1946, and in material collected by personnel of the Office of Biology in 1939. One of these larvae was found encapsulated on the external

surface of the stomach wall, others were found in the flesh, the ovaries, the pyloric caeca, the swim bladder, and the body cavity. They were collected from fish from lakes and rivers of the Chicoutimi, Jacques-Cartier, and Ste-Anne-du-Nord drainage systems.

The worms are cylindrical, brownish in color, 25 to 34.5 mm. in length. The cuticle is thick and transversely striated. The anterior extremity when viewed laterally is conical in outline; *en face* view of the head shows that the mouth opening is elongated dorsoventrally and flanked by two trilobed pseudolabia of which the lateral lobes are the larger. There are four submedian papillae located on the base of the pseudolabia. This arrangement suggests that the dorsodorsal-laterodorsal and ventroventral-lateroventral papillae have become fused. The internal circle of papillae is apparently absent.

Cervical papillae are present, located 0.037 to 0.04 mm. from the anterior extremity. The width of the anterior extremity at the level of the cervical papillae is 0.131 to 0.149 mm. The mouth is followed by a single vestibule, 0.125 mm. in length by 8μ in diameter. The oesophagus following the pharynx is from 8 to 9 mm. long and divided into an anterior muscular portion and a posterior glandular portion that is about six times as long as the muscular one. Both parts of the oesophagus are traversed by a strong oesophageal tube. The intestine is irregular in outline and difficult to follow; it terminates in a chitinous rectum averaging 0.725 mm. in length. At the level of its junction with the intestine, conspicuous glands could be seen. The anus is terminal. Neither the excretory pore nor the nerve ring was seen in any of the specimens. In two of the immature forms there were traces of growth of the male genital rudiment.

The structure and characters of the cephalic extremity are considered to be sufficient to class these immature forms as Spiruridae and, more specifically, as being representative of the subfamily Spirurinae as defined by Chitwood and Wehr (2).

Acanthocephala

A single species belonging to the genus *Echinorhynchus* was found. It was tentatively identified as *E. lateralis* Leidy, 1851, from the description given by Richardson (14) of forms he found in speckled trout in Lake Edward, Que. Some of the present specimens were sent to Prof. Van Cleave who found them (18) to be identical with specimens that Richardson had previously sent him.

This species is widely distributed in the area and was recorded from all the drainage systems studied. They were found in 55 of the 210 fish examined; they were chiefly in the intestine although many were also in the pyloric caeca.

References

1. BANGHAM, V. and VENARD, C. E.   Parasites of fish of Algonquin Park lakes.   Publications of the Ontario Fisheries Research Laboratory, 65 : 33-46.   1946.
2. CHITWOOD, B. G. and WEHR, E. E.   The value of cephalic structures as characters in nematode classification, with special reference to the superfamily Spiruroidea.   Z. Parasitenk. 7 : 273-335.   1934.

3. CHANDLER, A. C. New genera and species of nematode worms. Proc. U.S. Natl. Museum, 78 (Art. 23) : 1-11. 1931.

4. CHOQUETTE, L. P. E. *Phyllodistomum lachancei* sp. nov., a trematode from the ureters of *Salvelinus fontinalis* (Mitchill), with a note on its pathogenicity. Can. J. Research, D, 25 : 131-134. 1947.

5. HOPKINS, H. S. The papillose Allocreadiidae. Ill. Biol. Monographs, 12 : 1-80. 1934.

6. HUNNINEN, A. V. and HUNTER, G. W. III. On the species of *Crepidostomum* in trout. Trans. Am. Microscop. Soc. 52 : 150-157. 1933.

7. KUITUNEN-EKBAUM, E. A study of the cestode genus *Eubothrium* of Nybelin in Canadian fishes. Contrib. Can. Biol. Fisheries, 8 : 90-98. 1933. •

8. LYSTER, L. L. Parasites of freshwater fish. II. Parasitism of speckled and lake trout and the fish found associated with them in Lake Commandant, Que. Can. J. Research, D, 18 : 66-78. 1940.

9. LYSTER, L. L. *Apophallus imperator* sp. nov., a heterophyid encysted in trout, with a contribution to its life history. Can. J. Research, D, 18 : 106-121. 1940.

10. MACLULICH, D. A. *Proteocephalus parallacticus*, a new species of tapeworm from lake trout, *Cristivomer namaycush*. Can. J. Research, D, 21 : 145-149. 1943.

11. MACLULICH, D. A. Parasites of trout in Algonquin Provincial Park, Ontario. Can. J. Research, D, 21 : 405-412. 1943.

12. MILLER, M. J. Parasites of freshwater fish. III. Further studies on the internal trematodes of fish in the central St. Lawrence watershed. Can. J. Research, D, 18 : 423-434. 1940.

13. MILLER, M. J. A critical study of Stafford's report on "Trematodes of Canadian Fishes" based on his trematode collection. Can. J. Research, D, 19 : 28-52. 1941.

14. RICHARDSON, L. R. Observation on the parasites of the speckled trout in Lake Edward, Quebec. Trans. Am. Fisheries Soc. 66 : 343-356. 1936.

15. SKINKER, M. S. Three new parasitic nematode worms. Proc. U.S. Natl. Museum, 79 (Art. 24) : 1-9. 1932.

16. STAFFORD, J. Trematodes from Canadian fishes. Zool. Anz. 27 : 481-495. 1904.

17. VAN CLEAVE, H. J. and MUELLER, J. F. Parasites of Oneida Lake fishes. Part 3. A biological and ecological survey of the worm parasites. Roosevelt Wild Life Ann. 3 : 1-334. 1934.

18. VAN CLEAVE, H. J. Personal communication. 1947.

19. WARDLE, R. A. The cestoda of Canadian fishes. II. The Hudson Bay drainage system. Contrib. Can. Biol. Fisheries, 7 : 379-403. 1932.

THE FUNCTION OF THE GIANT AXON OF *MYXICOLA INFUNDIBULUM* MONTAGU[1]

By J. A. Colin Nicol[2]

Abstract

The polychaete, *Myxicola infundibulum*, contains a very large nerve fiber that runs throughout the nerve cord and gives off peripheral branches to the longitudinal muscles. Movements of the animal are quick synergic contractions of the whole body and slower metachronous locomotory movements. Injury to the giant axon without interrupting the rest of the nerve cord blocks the passage of the quick contraction but not of slower locomotory waves. It is concluded that the quick end-to-end shortening is intermediated by the giant axon and that slow waves of movement depend upon transmission through short segmentally linked neurones. Traction of one segment on another is not effective in transmitting either type of movement. The giant fiber response is of an all-or-none nature. Repetitive stimuli lead to summation of muscular contractions. The axon conducts in either direction during the natural life of the animal. The nature of the effective stimuli, the simplicity of the neuronal arrangement involved, and the character of the synergic response are discussed in terms of their survival value to the species.

Introduction

There are a considerable number of experimental studies dealing with the giant nerve fibers of lumbricids, and certain aspects of their function are now well known, but few investigators have concerned themselves with the functioning of the giant fibers of polychaetes. Friedländer (11) suggested that the three dorsal giant axons of the earthworm were involved in the 'startle' reaction or end-to-end shortening in which the animal suddenly contracts in length when disturbed and this hypothesis has since been confirmed by several investigators. Bovard (2) made a rather ingenious approach to the problem when he showed that following section of the nerve cord of the earthworm the giant axons regenerated and grew together more slowly than the rest of the cord. Correlated with this fact he found a corresponding delay in the return of quick end-to-end contractions throughout the length of the worm, in contrast to the quicker return of co-ordination of slower locomotory movements. Yolton (27), Stough (25), and ten Cate (26) subsequently confirmed these observations by sectioning the giant axons of the earthworm without interrupting the rest of the nerve cord, and they showed that the end-to-end contraction failed to pass from one part of the body to the other across the lesion, thus clearly indicating that the giant nerve fibers intermediate this response.

The giant axons of many polychaetes attain even greater dimensions than in oligochaetes and, moreover, some species of polychaetes show the same kind of end-to-end shortening as the earthworm. It has been suggested frequently, therefore, that the giant axons of polychaetes have the same

function as those of the earthworm but no conclusive evidence has ever been presented for this correlation. I have recently made a study of the structure of the giant nerve fibers of several species of sabellids (18) and in the course of that investigation some evidence was obtained for the functioning of the giant axon of *Myxicola infundibulum* Montagu. The several aspects of the problem that presented themselves may be treated as follows: (1) does the giant axon of *Myxicola* intermediate the quick end-to-end contraction of this animal; (2) is it concerned with any other movements of the animal; (3) what is its position in the nervous pathway in which it occurs and does it possess any physiological polarity in the intact animal comparable to the law of forward direction of vertebrate neurones?

Structure of the Giant Axon of *Myxicola*

Several authors have commented on the extraordinarily large size of the giant axon of *Myxicola infundibulum* (7, 8, 21) and these earlier accounts have been confirmed in part and augmented by recent communications (18, 19). A single large axon runs throughout the length of the nerve cord, beginning in the suboesophageal ganglion in setiger II. This axon subdivides into two fibers on several occasions in the first few segments (setigers II to IV) and sends a branch up each oesophageal connective into the supraoesophageal ganglia where the two branches terminate, independently of each other, in a pair of relatively large nerve cells. The giant axon also is connected with numerous nerve cells throughout its length in the nerve cord and since it is a continuous structure without internal dividing septa or 'macrosynapses' it constitutes a syncytial neurone. It varies considerably in size in different animals and in different states of contraction or extension, having a greater diameter in larger individuals, and increasing about twofold in diameter during contraction and shortening of the worm. It also varies in diameter along its length. In mature specimens representative axon diameters are about 500 to 1000μ in the thorax and anterior abdomen, followed by a gradual decrease to 100μ or less in the posterior abdomen. A transverse section through the anterior third of the body reveals that nearly all the nerve cord is occupied by the giant nerve fiber and it constitutes about 33% of the entire volume of the cord. About eight large peripheral branches arise from the nerve cord in each segment and these peripheral branches enter the body wall and extend towards the mid-dorsal line, giving off small collaterals along their course to the longitudinal muscle fibers. The giant fiber branches run parallel to one another without apparent anastomoses in the periphery of the body. The giant axon, therefore, is not only a large syncytial structure lying within the nerve cord, but it also extends into the body wall and envelops and penetrates the entire longitudinal musculature of the animal (Figs. 1, 2, 3).

The longitudinal muscles of *M. infundibulum* are very strongly developed and form four massive and symmetrically arranged areas, two dorsal and dorsolateral, separated from each other by the dorsal medial mesentery, and two ventrolateral, separated by the median nerve cord.

Behavior of *M. infundibulum*

M. infundibulum is a cosmopolitan species about 13 cm. long.   It consists of a ciliated feeding crown at its anterior end and a trunk of about 130 segments bearing short chaetae and uncini but no parapodia.   It lives in a bulky mucous tube buried in sand and clay near low tide mark and extends its crown and anterior segments from its tube in order to feed.
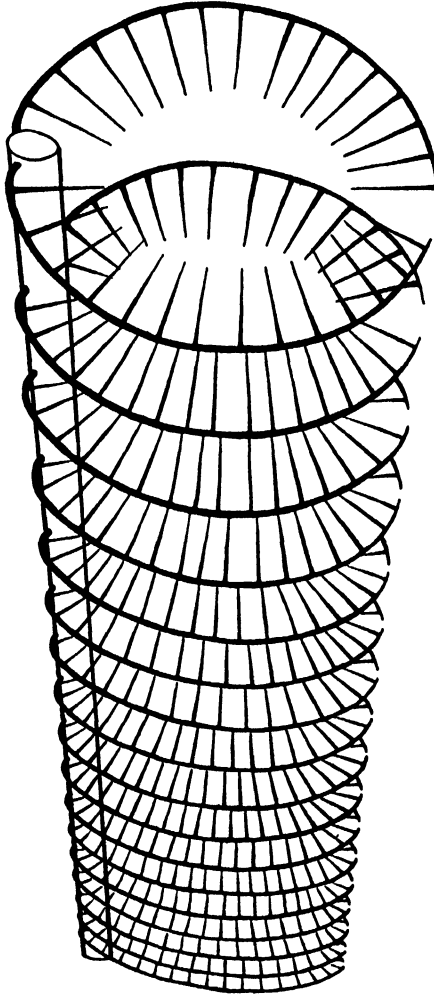
FIG. 1.   *Diagram of the giant axon of Myxicola infundibulum, showing peripheral branches that extend into the body wall.*

The surprisingly quick and powerful contraction of this species has been noted by a number of observers (6, 17) and Friedländer (11) stated that it showed very little activity apart from this quick shortening.   He noted, moreover, that when the nerve cord was cut across the quick contraction

PLATE I
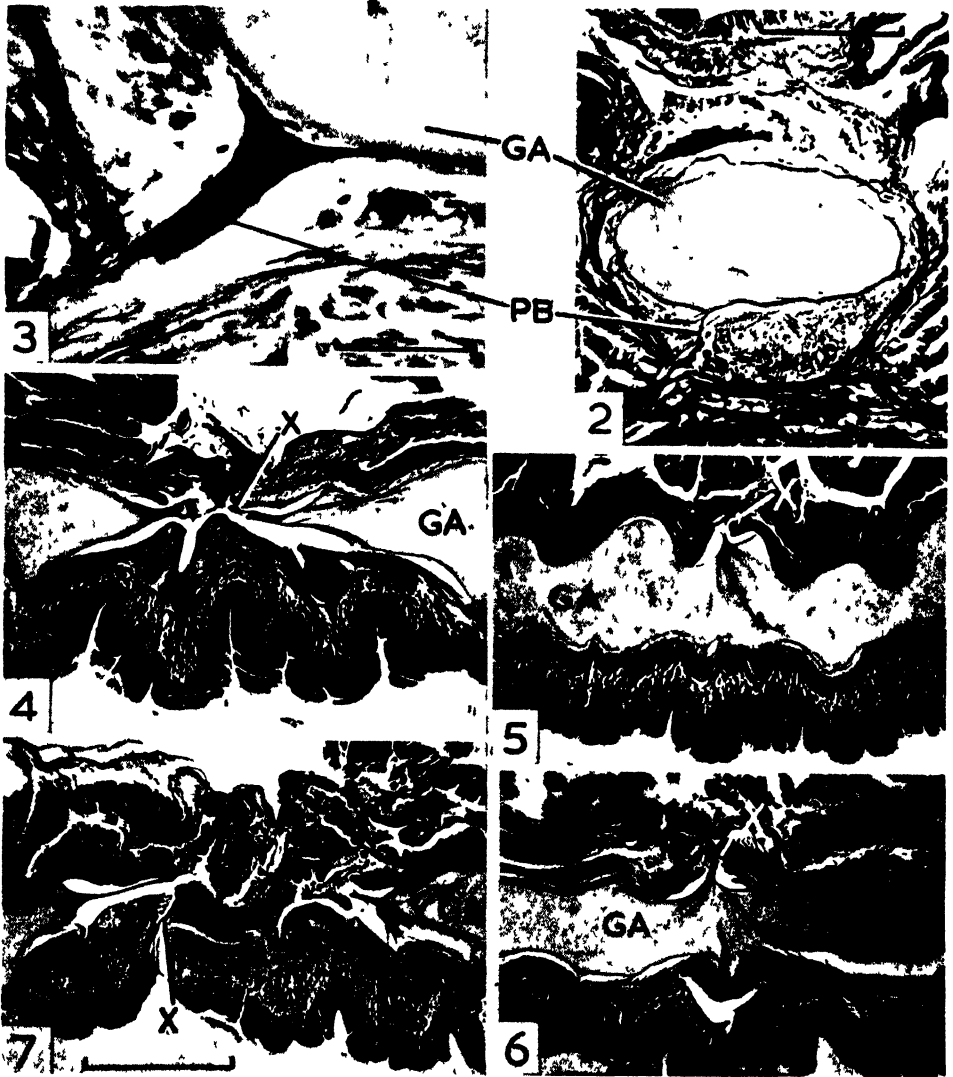
FIGS 2 to 7   *Photographs of sections through the nerve cord and giant axon of Myxicola infundibulum*

FIG. 2.   *Transverse section through the nerve cord, showing a peripheral branch arising from the left ventrolateral region of the giant axon*   FIG. 3   *A more highly magnified view of the origin of a peripheral branch.   Silver impregnation (Holmes' method).*   FIGS. 4 to 7 *Longitudinal sections through the nerve cord of operated animals.   Ehrlich's haematoxylin and eosin.*

Legend —GA, giant axon.   PB, peripheral branch of giant axon.   X, site of transection of giant axon (and nerve cord).

Magnifications indicated by horizontal lines: in Figs 2 and 3, equivalent to 250 µ, in Figs. 4 to 7, equivalent to 1 mm. (shown on Fig. 7 only).

did not pass the injured segment to the part of the body beyond, but since the smaller fibers of the neuropil as well as the giant axon are transected in such an operation it does not in itself offer proof of the function of the giant axon.

If a worm is removed from its tube and placed in a dish of sea water it slowly elongates and opens its crown. If any part of the body is touched it gives a single quick contraction and closes its crown. If subjected to a series of tactile stimuli it responds by a series of quick contractions, one per stimulus, and vigorous and repeated tactile stimuli will cause the animal to shorten to about one-third of its extended length into a short and rigid cone. The animal shows some kind of adaptation to tactile stimulation since it ceases to give quick contractions after about the eighth stimulus. On cessation of stimulation, subsequent elongation of the worm and opening of the crown occupy from 5 to 10 min.

The animal also shows several other types of movement. In a dish containing sea water it slowly moves backwards by means of antiperistaltic waves originating at the posterior end; the tail also is in constant exploratory movement, slowly moving about the substratum. On a floor of sand it initiates burrowing by slow forward and backward movements of its tail and soon covers itself with sand. Slow peristaltic waves are rather difficult to follow by eye but they can be accentuated and clearly watched following chemical stimulation. When a drop of concentrated hydrochloric acid is dropped into the water above the tail of the animal it gives a series of quick contractions, followed by a succession of antiperistaltic waves of thickening and thinning originating near the posterior end and passing forwards over the posterior third of the animal. As a result the posterior end is kept retracted from the region of stimulation. When a drop of acid is dropped above the crown, on the other hand, the animal gives one or more quick contractions, the anterior half of the body is kept retracted, but the posterior end soon elongates and begins exploratory movements. The result, again, is that the stimulated region is retained away from the source of stimulation. Swimming and lashing movements have never been observed, although they do occur rarely in the allied species, *Branchiomma vesiculosum* Montagu.

The quick shortening can be elicited by several types of stimuli. The first one that has been mentioned is tactile stimulation. Touching any part of the body, say with a blunt seeker, causes the animal to contract. The most sensitive areas to tactile stimulation are the crown, head, and tail while the intervening trunk appears to be less sensitive. Stimulation of the crown gives a rough guide to the intensity of stimulation necessary to evoke a response. Gentle depression of one of the pinnules is without effect or results in slight folding of the crown only; more vigorous deformation of one or more pinnules calls forth a quick contraction. The second type of stimulus causing a quick contraction is vibration of the substratum. Jarring the table, say by setting down another container, causes all the animals to contract and close their crowns. Currents created by agitating the water also lead to end-to-end

shortening. The third effective stimulus is some noxious chemical agent like strong acid. Finally, sudden change in light intensity occasionally elicits a quick shortening movement but this stimulus is not uniformly effective. It causes a response in only some of the animals in any one container and only occasionally and erratically in the case of any one specimen. In this respect *Myxicola* differs from some other species of sabellids and serpulids in which the light reflex is strongly developed, for example *Branchiomma vesiculosum* and *Hydroides dianthus* (16).

It is probable that the behavior of *Myxicola* is more complex than the responses described above would indicate but this description serves at least to delimit some of the characteristic behavioral patterns of the animal for immediate exploration. In general we find that the responses of *Myxicola* are of two types: (1) quick end-to-end synergic contractions of the massive longitudinal musculature; (2) slower metachronous locomotory waves. In the nervous system we find two sharply distinguishable categories of neurones: (1) a large continuous giant axon extending throughout the nerve cord; (2) small segmentally arranged neurones forming short pathways. Evidence will now be presented that the giant axon mediates the end-to-end contractions.

Effect of Cutting the Nerve Cord and the Giant Axon

Since the giant axon of *Myxicola* is so large an opportunity is afforded of transecting it easily without damaging the rest of the nerve cord and this operation has been performed upon several specimens. Healthy worms were chosen that exhibited the quick contraction throughout their length. The animal was cut open dorsally by a slit 1 to 2 cm. long, the gut removed from the exposed region and the body wall pinned out in a dissecting dish under sea water. The giant axon, clearly visible, was then cut across with a sharp-pointed scalpel, care being taken not to transect the underlying neuropil of the cord at the same time. Reactions of the animal were noted, after which the operated region was prepared for histological examination. The specimens were fixed in Bouin's fluid made up in sea water, double-embedded in celloidin-paraffin, and cut longitudinally at 15μ as serial sections, which were stained with Ehrlich's haematoxylin and eosin. The protocols of these experiments follow. In each case the giant axon was cut in the anterior abdominal region.

Specimens Nos. 601, 607, 610

In these three specimens the giant axon alone was transected. The following responses were observed after operation. Sharp mechanical stimulation of the anterior end resulted in a quick contraction that proceeded only as far as the injured region. But after a brief interval a progressive wave of thickening proceeded along the abdomen posterior to the injured region. On mechanical stimulation of the posterior end the region posterior to the lesion gave a quick contraction that did not extend in front of the operated region. A slow contraction sometimes occurred in the anterior region of the body

following posterior stimulation. Pinning down the body wall over a length of several segments in the operated region produced no change in the movements described.

Histological Picture

Sections showed that in each specimen the giant axon was injured while the ventral region of the cord was intact (Figs. 4, 5, 6). In each specimen axoplasm had escaped into the coelomic cavity. There were, however, individual differences in the extent of the injury to the giant axon. In only No. 610 was there complete division of the giant axon; in Nos. 607 and 608 the axon was only partially transected. It has been observed previously (18) that the axoplasm of the giant fiber of this species is viscous and shows little tendency to flow. Consequently, it is not surprising that a transverse cut should result in little loss of axoplasm and, in two specimens, only partial interruption of axoplasm.

No. 609

This specimen represents one of many in which the entire nerve cord was cut. Stimulation of either the anterior or posterior end gave rise to quick contractions, followed by slower waves of thickening. These movements were confined to the portion of the body stimulated, that is, they were always proximal to the lesion: in no case did either quick contractions or slower waves leap over the operated region.

Histological Picture

The giant fiber was interrupted and axoplasm had escaped into the coelomic cavity. In addition, the rest of the nerve cord and the body wall in the mid-ventral line were cut through (Fig. 7).

It may be concluded from these experiments that the giant axon is responsible for intermediating the quick shortening movement of *Myxicola* since interruption of the axon alone blocks the passage of this contraction across the injured region. The experiments show also that the nerve cord is concerned with the passage of slow metachronous movements along the length of the animal and that transmission of these movements cannot take place by pull or traction of one segment or region on successive segments.

Effect of Cutting the Lateral Body Wall

The following operation was performed to determine whether there are any anastomoses among the branches of the giant axon peripherally in the body wall. The ventral body wall was cut through longitudinally on one side, over a length of 5 to 10 segments, parallel to the nerve cord. The animal was then stimulated mechanically to cause the giant fiber response. Longitudinal contraction normally leads to shortening and swelling of each segment. The resultant quick contraction after this operation involved the entire length of the body with the exception of the body wall lateral to the incision. This was apparent from the fact that the animal curved outwards during contraction

towards the injured side.   Dorsal to the cut the segments remained expanded
and became narrower towards the mid-dorsal line.   Since the peripheral
branches of the giant axon were cut ventrally by this operation but were left
intact dorsally, it may be concluded that these peripheral axons do not fuse
with one another either dorsally, across the mid-line of the body, or longi-
tudinally, successive branches with one another.   These observations support
the appearance seen in histological sections in which each peripheral branch
of the giant axon remains discrete in the body wall and forms a half-ring on
one side of the body (Fig. 1).

Giant Axon Contractions

A preliminary analysis of giant axon contractions of *Myxicola* has been
made, using electrical stimuli.   Condenser discharges were used and stimuli
were applied by Ag–AgCl electrodes (14).   The latter were suspended in glass
tubing containing sea water.   The narrow mouths of the tubes, about 4 mm.
apart, were placed on the skin of the animal beneath the surface of the sea
water.   Kymograph records were made for purpose of measurement.

A single electrical stimulus, above threshold, causes a single vigorous con-
traction of the animal.   This sharp contraction occupies about two seconds
and is followed by slow elongation, sometimes interrupted by small contractile
waves.   A contraction may be produced by stimulating any part of the body
except the distal half of the branchial crown.   This suggests that the current
is stimulating the giant axon directly and not via peripheral sensory neurones
since the giant axon terminates in the supraoesophageal ganglia but can be
fired by mechanical stimulation of the branchial crown.   Moreover, if the
anterior half of the animal is anaesthetized by immersion in 6% magnesium
chloride for 15 min., tactile stimulation of this region is usually ineffective
while an electrical stimulus always causes a quick contraction.

A series of spaced stimuli (from 1 to 40 stimuli per minute) produces a
series of discrete contractions, one per stimulus.   Stimulation at any one
position over a given period (usually about 10 min. gives a series of consistent
results) shows that the shortening response is of an all-or-none nature.   Under
these conditions and with constant potentiometer setting, successive contrac-
tions are nearly the same height.   When stimulation is subthreshold there is
no movement apart from slight swelling of the body wall immediately below
the electrodes.   A threshold stimulus produces one contraction while increase
in stimulus strength produces no change in the height or type of response.
Repetitive threshold or suprathreshold stimuli, at increasing frequency, result
first in clonus- and then in tetanus-like contractions.

Although single subthreshold stimuli (frequency one every 10 sec.) are
ineffective in causing a contraction, it is possible to obtain summation of two
individually inadequate stimuli by excitation at shorter intervals.   The range
explored was 0.56 to 10 sec. intervals, of which 0.56 sec. to five seconds were
effective in summation.   The long effective period of facilitation (up to five
seconds) with two subthreshold stimuli would seem to indicate that summation

can occur in the epidermal receptors (not discounting the possibility that summation occurs in the giant axon itself at shorter intervals). This observation, in conjunction with the necessity of applying vigorous tactile stimuli to the pinnules of the crown, mentioned above, suggests that the giant axon can be fired normally as the result of temporal and spatial summation in the afferent pathways.

Discussion

The giant axon of *Myxicola* conducts impulses causing the quick end-to-end contraction of the entire animal and therefore has a function similar to that of the three giant axons of the earthworm but it differs from the latter in one important respect, viz., in its lack of functional polarity. In the earthworm, despite the presence of segmentally arranged macrosynapses, the giant axons are capable of conducting in either direction as the result of electrical stimulation (9) but in the intact animal the median giant usually conducts impulses largely anteroposteriorly following tactile stimulation of the anterior end of the body and the two laterals posteroanteriorly following tactile stimulation of the posterior region (5, 23, 25). Rushton (23) and Stough (25) believe that this difference in conduction direction results from differences in the sensory connections of the median and lateral fibers in the anterior and posterior regions of the worm. Rushton (22) found significant differences in the kind of end-to-end contractions resulting from anterior and posterior stimulation, respectively. Bullock (5) has shown also that in *Neanthes virens*, which has five giant axons (15), the giant fibers conduct equally well in either direction along the length of the worm as the result of electrical stimulation. In this species, however, the median giant fiber is fired by tactile stimuli in the anterior quarter, the pair of mediolateral giant fibers by tactile stimuli in the posterior three-quarters, and the two large lateral giant axons by stronger stimuli at all levels. The giant fiber system of *Neanthes* thus resembles that of *Lumbricus* in certain features of functioning as well as in the presence of intersegmental macrosynapses (24). Since the giant axon of *Myxicola* is a continuous structure, no complications are introduced by the presence of transverse septa and *ex hypothesi*, from the bases of the neurone doctrine, it would be expected to conduct an impulse, originating in any part of its length, throughout its extent in the nerve cord and body wall. It is of interest, however, that it is capable of being excited by tactile stimulation in any part of the body and consequently transmits impulses in either direction along its length in the natural life of the animal.

Afferent fibers from the coronary nerves enter into the formation of the dense neuropil which surrounds the two giant fiber nerve cells in the supra-oesophageal ganglia, and afferent fibers from the epidermal receptors in each segment form a complex feltwork immediately beneath the giant axon in the nerve cord. The histological picture suggests that the reflex arc involved in the giant fiber response is a comparatively simple one, of sensory neurones making direct contact with the giant axon, and of the giant axon itself constituting the efferent side of the arc. The giant fiber, in its role of an efferent

axon traversing the entire central nervous system and directly innervating all the longitudinal muscle fibers of the trunk, constitutes a unique example of an initial and a final common path for a synergic response. The two most striking features of this arrangement are (1), the simplicity of the afferent path in which sensory neurones, with short centripetal course, are capable of firing the giant axon at all points and (2), the simplicity of the efferent side of the arc with a single long distance fiber lying in the central nervous system and itself directly innervating the major muscle mass of the body.

There is no evidence that the giant axon is concerned with any other type of movement besides the shortening reaction of the animal since variation in intensity and frequency of stimulation produces either one or a series of similar sharp contractions. The giant axon is not necessary for the propagation of slower locomotory and contractile waves since these are not stopped by section of the giant axon but are interrupted by section of the entire nerve cord. In the earthworm slow locomotory waves can be transmitted along the body of the animal even when pieces of the cord are removed, by traction of one segment on another (1, 10, 12). Quick end-to-end contractions are not transmitted by this means in earthworms. In *Nereis* on the other hand (3, 13), section of the nerve cord abolishes all co-ordination of locomotion between the two halves of the body. *Myxicola* resembles *Nereis* in this regard in that pull of one segment on another is not effective in transmitting the quick contraction or locomotory waves along the body. The actual rate at which these slow waves of thickening and thinning are propagated has not been measured but they can be followed easily with the eye as they course slowly over the surface of the animal; their velocity is very slow and is of the order of a few centimeters per second. Bovard (1) gives a mode of 25 mm. per second for the transmission of locomotory impulses in the earthworm. The low rate in *Myxicola* suggests that transmission is effected by means of neuronal chains, with consequent synaptic delay in each segment, and not by continuous axons extending long distances in the cord.

Confirmation of some of these results has since been obtained by Nicol *et al.* (20, pp. 243-244) who have recorded giant fiber action potentials from the dissected nerve cord and from intact specimens of *Myxicola*. In this investigation it has been found that the giant axon conducts in either direction as the result of electrical and tactile stimulation and that single stimuli, electrical or tactile, give rise to single action potentials, one for each stimulus. Each giant fiber action potential is followed in turn by a single muscle potential, corresponding to the synergic contraction of the longitudinal muscles of the trunk (making allowance, of course, for conduction time). These observations supplement those reported in the present communication in that they demonstrate that each quick contraction of the animal is caused by a single all-or-none impulse in the giant axon.

It is possible to recognize many features of survival value to the animal in the arrangement and functioning of the giant fiber system of *Myxicola*. The saving of conduction time that results from the large diameter of the axon

and the continuity of the efferent pathway has already been noted (18). *Myxicola* is a comparatively inactive, sedentary species that usually exposes its anterior end only above the substratum. Approach of predators would be signalled by touch, vibration of the ground, water currents, and passing shadows, all stimuli to which the animal is sensitive in varying degree and to which it responds by a single contraction that jerks it back into its tube. Weak or subthreshold stimuli, at short enough interval to cause summation, would also cause its withdrawal, and strong repeated stimuli would result in successive quick contractions and further shortening. Since the animal elongates again rather slowly after a giant fiber response it would appear that short segmental neurones take over after each giant axon discharge and maintain the animal in a state of tonus from which it is only slowly released, thereby retarding exposure of the head and the crown for a considerable interval.

As far as is known, all sabellids and serpulids possess well-developed giant axons and exhibit quick reflex shortening similar to that observed in *Myxicola*. In *Sabella*, at least, the two giant fibers anastomose by transverse branches in each segment to form a composite giant axon and they probably function as a unit as in *Myxicola*. However, since the giant axons of polychaetes display the most diverse form and arrangement in species of different families, and even within a family, caution should be exercised in describing their function until more is known regarding the behavior of various representatives of this order. Other types of synergic contractions apart from symmetrical end-to-end shortening have been described in several polychaete species, and the possibility that giant axons may be involved in some of these contractions should not be discounted.

Acknowledgments

References

1. BOVARD, J. F. The transmission of nervous impulses in relation to locomotion in the earthworm. Univ. Calif. Pub. Zool. 18 : 103-134. 1918.
2. BOVARD, J. F. The function of the giant fibers in earthworms. Univ. Calif. Pub. Zool. 18 : 135-144. 1918.
3. BUDDENBROCK, W. v. Grundriss der vergleichenden Physiologie. Gebrüder Borntraeger, Berlin. 1928.
4. BULLOCK, T. H. Functional organization of the giant fiber system of *Lumbricus*. J. Neurophysiol. 8 : 55-71. 1945.

5. BULLOCK, T. H.   Organization of the giant nerve fiber system in *Neanthes virens*.   Biol. Bull. 89 : 185-186.   1945.

6. CLAPARÈDE, E.   Les annélides chétopodes du Golfe de Naples.   Mém. soc. phys. Genève, 20 : 365-542.   1870.

7. CLAPARÈDE, E.   Recherches sur la structure des annélides sédentaires.   Mém. soc. phys. Genève, 22 : 1-200.   1873.

8. CUNNINGHAM, J. T.   On some points in the anatomy of Polychaeta.   Quart. J. Micr. Sci. 28 : 239-278.   1887.

9. ECCLES, J. C., GRANIT, R., and YOUNG, J. Z.   Impulses in the giant nerve fibres of earthworms.   J. Physiol. 77 : P23-P25.   1933.

10. FRIEDLÄNDER, B.   Ueber das Kriechen der Regenwürmer.   Biol. Zentr. 8 : 363-366. 1888.

11. FRIEDLÄNDER, B.   Über die markhaltigen Nervenfasern und Neurochorde der Crustaceen und Anneliden.   Mitt. Zool. Sta. Neapel, 9 : 205-265.   1889.

12. FRIEDLÄNDER, B.   Beiträge zur Physiologie des Centralnerversystems und des Bewegungsmechanismus der Regenwürmer.   Pflügers Arch. ges. Physiol. 58 : 168-206. 1894.

13. GRAY, J.   Studies in animal locomotion.   VIII. The kinetics of locomotion of *Nereis diversicolor*.   J. Exptl. Biol. 16 : 9-17.   1939.

14. HALL, D. M. and PANTIN, C. F. A.   The nerve net of the Actinozoa.   V. Temperature and facilitation in *Metridium senile*.   J. Exptl. Biol. 14 : 71-78.   1937.

15. HAMAKER, J. I.   The nervous system of *Nereis virens* Sars.   A study in comparative neurology.   Bull. Museum Comp. Zoöl. Harvard, 32 : 87-124.   1898.

16. HARGITT, C. W.   Observations on the behavior of tubicolous annelids.   III. Biol. Bull. 22 : 67-94.   1912.

17. MONTAGU, G.   Description of several marine animals found on the south coast of Devonshire.   Trans. Linnean Soc. London, Zool. 9 : 81-114.   1808.

18. NICOL, J. A. C.   The giant nerve fibres in the central nervous system of *Myxicola* (Polychaeta, Sabellidae).   Quart. J. Micr. Sci. 89 : 1-45.

19. NICOL, J. A. C. and YOUNG, J. Z.   Giant nerve fibre of *Myxicola infundibulum* (Grube).   Nature, 158 : 167.   1946.

20. NICOL, J. A. C., SMYTH, C. N., and WHITTERIDGE, D.   Conduction velocity in relation to axon diameter in *myxicola infundibulum*.   Abstracts of Communications.   XVII Intern. Physiol. Congr., Oxford.   1947.

21. PRUVOT, G.   Recherches anatomiques et morphologiques sur le système nerveux des annélides polychètes.   Arch. zool. exptl. gén. 3 : 211-336.   1885.

22. RUSHTON, W. A. H.   Motor response from giant fibres in the earthworm.   Nature, 156 : 109-110.   1945.

23. RUSHTON, W. A. H.   Reflex conduction in the giant fibres of the earthworm.   Proc. Roy. Soc. London, B, 133 : 109-120.   1946.

24. STOUGH, H. B.   Giant nerve fibers of the earthworm.   J. Comp. Neurol. 40 : 409-463. 1926.

25. STOUGH, H. B.   Polarization of the giant nerve fibers of the earthworm.   J. Comp. Neurol. 50 : 217-230.   1930.

26. TEN CATE, J.   Sur la fonction des neurochordes de la chaîne ventrale du ver de terre (*Lambricus terrestris*).   Arch. néerl. physiol. 23 . 136-140.   1938.

27. YOLTON, L. W.   The effects of cutting the giant fibers in the earthworm, *Eisenia foetida* (Sav.).   Proc. Nat. Acad. Sci. 9 : 383-385.   1923.

AN UNUSUAL MASKINONGE FROM LITTLE VERMILION LAKE, ONTARIO[1]

By G. S. CAMERON[2]

Abstract

An unusual type of maskinonge found in two lakes in Kenora District, Ontario, is regarded as a hybrid between *Esox masquinongy* and *Esox lucius*. It differs from the typical maskinonge found in the same waters in having a stouter body, longer and deeper head, longer maxillary, and longer fins. It retains dark vertical bars throughout life whereas in the typical form these break up and tend to disappear with age. Of 69 specimens examined, six were of the presumed hybrid type. These all appeared to be sterile. They showed the following *Esox lucius* characters—cheeks totally scaled, head concave interorbitally, cheeks and opercula vividly marked.

The presence of an unusual type of maskinonge in Little Vermilion Lake, Kenora District, Ontario, was brought to general attention in 1945, when it was described as a new species, *Esox amentus*, by Godfrey (3).

During the summer of 1946, two months were spent on Little Vermilion Lake and a number of other lakes in the vicinity in connection with a taxonomic study of maskinonge undertaken by the Royal Ontario Museum of Zoology with the financial support of the Carling Conservation Club. The accompanying map indicates the location of these lakes, which drain by way of the English River, the Winnipeg River, Lake Winnipeg, and the Nelson River into Hudson Bay.

In the course of these studies, 69 specimens of maskinonge from Little Vermilion Lake, and a smaller connecting lake, known as Maskinonge Lake (Musky Lake), were studied. The study included the making, on each specimen, of 28 measurements of such body proportions as head length, head depth, diameter of eye, length of snout, length of maxillary, body depth and width, caudal peduncle depth and length, and height and base of dorsal, anal, pectoral, and ventral fins. In addition, counts were made of scales in the lateral line, of branchiostegals, and of fin rays. Measurements and counts were made as described by Dymond (1). A description, including a photograph, was made of the markings and color pattern of each specimen. Age was determined also, by scale examination.
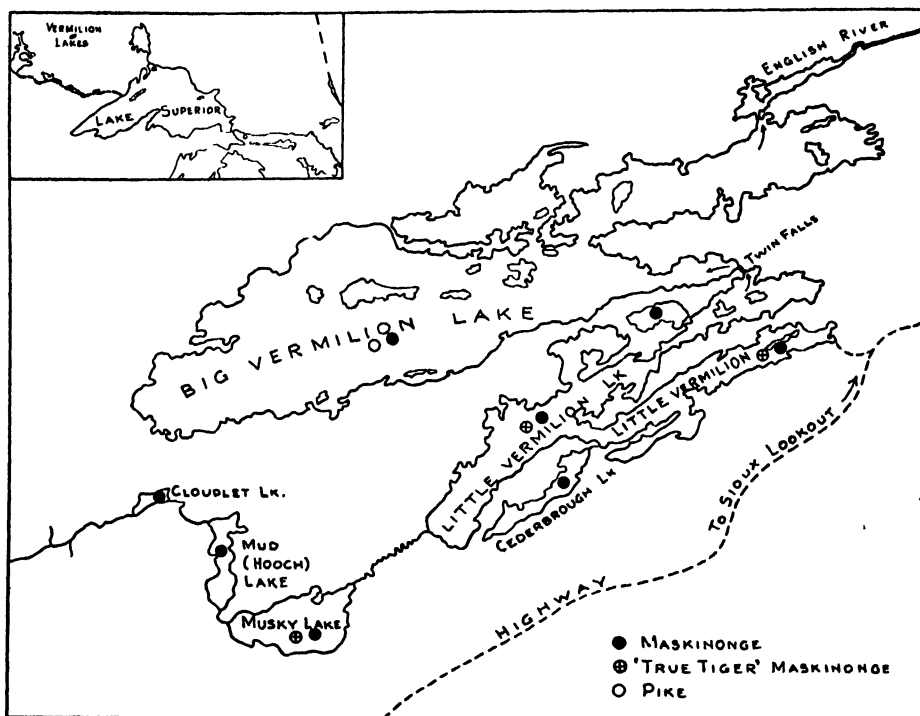
Map of Little Vermilion and surrounding lakes.

Table I presents a comparison of the body proportions and counts of the common and of the so-called 'true tiger' or *amentus* maskinonge. In the case of the common type, only average and extreme ranges are given.

A comparison between a number of these body proportions in the two types is presented graphically in Figs. 1, 2, and 3.

The table and figures indicate several significant differences between the common or typical maskinonge of Little Vermilion and Maskinonge lakes and the so-called 'true tiger' (*amentus*) variant, occurring in the same waters. As compared with the typical form, the variant has a much stouter body (deeper and wider in proportion to length), with a longer and deeper head, much more sharply concave interorbitally, longer maxillary (reaching a vertical through the posterior margin of the eye), and a caudal peduncle both shorter and deeper. The fins are all longer, with larger bases, while the scale count seems slightly lower. Other differences include the complete scaling of the cheek of the variant as compared with the naked lower half of the cheek of the typical form.

The color and markings of the two forms are quite different. Small specimens of the typical form (up to about 30 in. in length) are predominantly bluish green on the sides with distinct dark vertical bars (Fig. 4). Larger fish show a gradual darkening of color, while the markings become gradually

TABLE I

COMPARISON OF BODY PROPORTIONS AND COUNTS OF SCALES AND BRANCHIOSTEGALS OF THE COMMON TYPICAL MASKINONGE OF LITTLE. VERMILION AND MASKINONGE LAKES AND OF THE SO-CALLED 'TRUE TIGER' OR *amentus* TYPE FOUND IN THE SAME LAKES

All body proportions listed are expressed as thousandths of standard length; standard length in mm.

	Common type		'True tiger' (*amentus*) type						
	Average	Range							
Field number	—	—	037	024	075	042	050	035	Mean
Standard length	769	631-1022	850	862	885	904	908	911	887
Head length	276	252-309	315	321	329	303	315	324	318
Head depth	112	092-129	127	115	138	125	143	122	128
Eye	029	024-032	027	028	030	029	026	025	028
Snout	113	104-126	137	137	142	140	142	142	140
Interorbital	067	061-074	072	074	073	075	072	073	073
Maxillary	132	109-145	166	166	167	167	168	170	167
Snout to occiput	190	183-201	222	219	229	226	225	228	225
Body depth	183	163-222	188	209	206	204	193	200	200
Body width	105	089-122	108	115	120	122	120	113	116
Caudal peduncle									
length	124	103-147	108	122	119	132	123	122	121
depth	074	063-083	075	075	082	076	085	076	078
Dorsal									
rays	22	19- 23	22	22	23	22	23	22	22
height	115	099-129	119	119	134	134	121	128	126
base	120	109-141	132	133	137	128	140	133	134
Anal									
rays	20	18- 22	21	20	20	20	21	21	20/21
height	114	096-130	119	120	134	127	112	122	122
base	099	090-120	104	104	110	108	098	094	104
Pectoral									
rays	18	16- 19	18	18	18	17	18	18	18
height	115	101-132	114	130	139	133	118	132	128
base	036	030-043	042	042	042	039	035	039	040
Ventral									
rays	12-13	12- 13	12	12	12	12	12	12	12
height	100	089-117	101	112	119	118	110	120	113
base	036	031-040	039	037	037	038	035	038	037
Scales	149	137-156	143	150	143	146	140	145	145
Branchiostegals	17-18	16- 19	19/18	19/20	18/19	18/17	18/17	18/18	17/18

obscured (Fig. 5). The back is often so dark a shade of olive green as to be almost black. This color shades down through bronze to sides that have a ruddy ground color. As a fish ages, the bars break up into obscure blotches, which remain more distinct in the caudal region (Fig. 6). In the largest specimens (over 40 in.) the sides are usually of a uniform dirty brownish color. The belly is usually white, although that of some young maskinonge is marked by faint dark patches. The fins are typically of a brownish color with obscure darker blotches; the fins are often of a vivid red color.

ᶦ The variants are given the name 'true tiger' because they possess permanent distinct dark crossbars (Fig. 7) traversing light-colored sides, which show a subtle bluish tint. This light color darkens dorsally through a purple hue to a back that is so deep a purple as to appear black. The bars arise from this

black back and slope downwards and forwards, occasionally being broken by
distinct dark spots.   These markings are sometimes described as 'worm-
tracks'.   The cheeks and opercula are covered with distinct dark blotches,
while the fins are less reddish than those of the typical form, and are faintly
spotted.
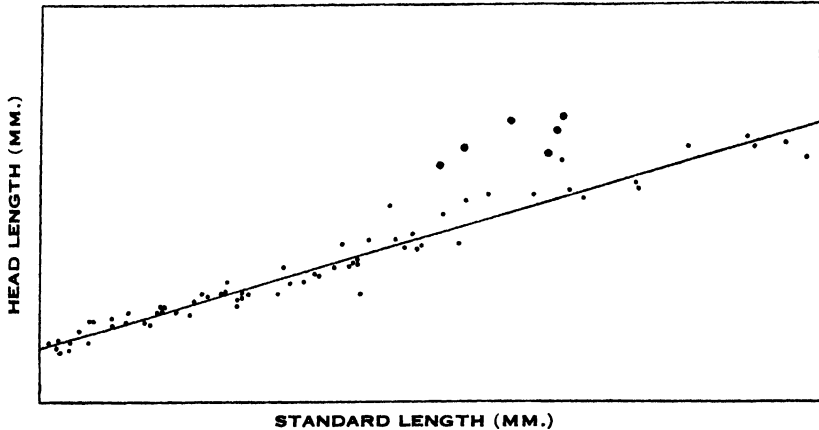
FIG. 1.   *Diagram showing relation between head length and standard length in typical
maskinonge (small dots) and 'tiger' maskinonge (large dots).*
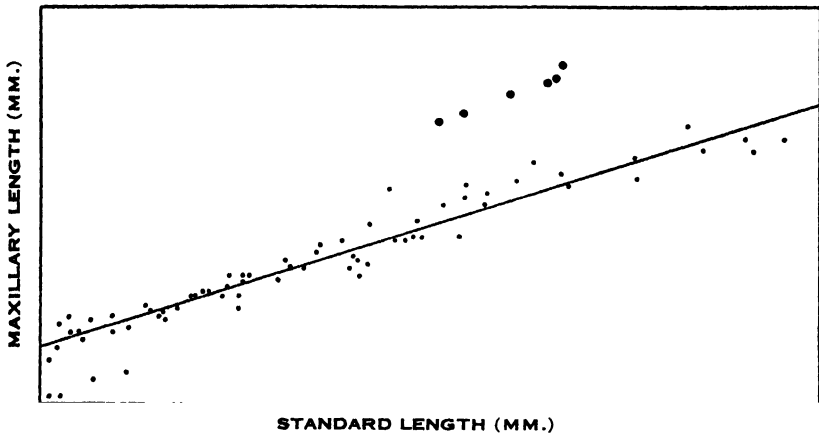
FIG. 2.   *Diagram showing relation between maxillary length and standard length in
typical maskinonge (small dots) and 'tiger' maskinonge (large dots).*

Through the co-operation of anglers fishing for maskinonge on Little
Vermilion and Maskinonge lakes, and local resort owners, a considerable
proportion of the specimens caught and retained were made available for
examination.   So keen are anglers to exhibit their catch of a rare 'true tiger'
that every specimen of this variant taken during the time the study was in
progress was photographed and examined.   The fact that of the 69 specimens
examined only six were of the 'true tiger' type indicates that this type is
comparatively rare.   This rarity, together with the striking beauty of the

fish makes it a prize eagerly sought after, and may in part explain its reputation for superior fighting qualities. Actually, experienced guides insist that both 'true tiger' and common maskinonge fight with equal vigor.
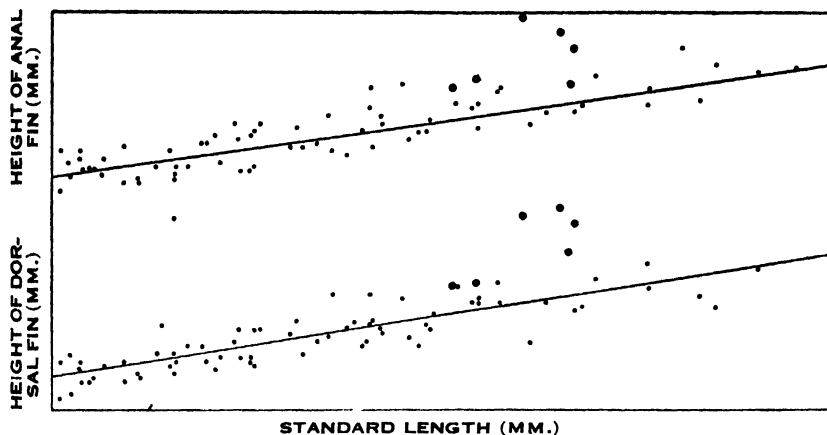
FIG. 3. *Diagram showing relation between height of anal and dorsal fins and standard length in typical maskinonge (small dots) and 'tiger' maskinonge (large dots).*

A striking feature of these variants was the fact that their gonads were so small and shrivelled as to suggest that they were nonfunctional. The texture was quite different from that of the gonads of normal specimens of the same size.

So far as is known this variant is confined to Little Vermilion and Maskinonge lakes although there were reports of its occurrence in Cliff and Height of Land lakes. Until specimens from these waters can be examined it will not be known whether these are of the same nature or merely vividly marked young of the typical form.

Several of the characters in which the so-called 'true tiger' maskinonge of Little Vermilion and Maskinonge lakes differ from the typical form suggests that it is a hybrid between the common maskinonge (*Esox masquinongy*) and the pike (*Esox lucius*). The pike is not known to occur normally in Little Vermilion and Maskinonge lakes, although it abounds in the lower neighboring lake, Big Vermilion, separated from Little Vermilion by a low falls. Little Vermilion and Maskinonge lakes are joined by a long meandering creek. At high water in spring when these fish spawn it is quite possible that occasional pike may gain entrance to the Maskinonge lakes above.

Some of the considerations that suggest that the 'true tiger' (*amentus*) maskinonge is a *masquinongy–lucius* hybrid are as follows.

It appears to be sterile.

It possesses the following characteristics of *Esox lucius*—cheeks totally scaled, head sharply concave interorbitally, cheeks and opercula vividly marked.

The scale count is intermediate between *lucius* and *masquinongy*.

Presumed *lucius–masquinongy* hybrids are known in other waters and have been produced artificially. Eddy and Surber (3) say that late-maturing pike have been reported as spawning with maskinonge and that evidence of hybridization has been found in the frequent appearance of specimens bearing maskinonge markings but having the cheeks entirely scaled as in the pike.

These authors further report that a large number of maskinonge eggs were successfully fertilized with pike milt at the Nevis Hatchery and that pike eggs were likewise successfully fertilized with maskinonge milt. Some of the resulting fish were reared in the vicinity of the Nevis Hatchery and some in tanks and ponds at the University of Minnesota.

Some of the characters shown by underyearlings of these hybrids have been reported by Eddy (2, pp. 25-27) as follows: "Both of the crosses were heavily barred. Some had the scales absent from the lower part of the cheek, but many showed the lower part of the cheek to be covered partially or entirely by scales." By Sept. 15 the hybrids were between 11 and 12 in. in standard length whereas the pure bred lunge were between 7 and 8 in. in standard length.

The heavy barring and the scaling on the lower part of the cheeks of the artificially produced hybrids correspond to the condition found in the presumed hybrid here reported.

The increased rate of growth and apparent infertility of the presumed hybrid correspond to the condition found by Hubbs and Hubbs (5) in the case of hybrid sunfish.

While the evidence for an increased growth rate in the case of the presumed hybrids reported here is not as great as in the case of the artificial hybrids during their first year there is some indication of it. The six specimens of the *amentus* type, ranging in standard length from 850 to 911 mm. were from 8 to 11 years of age, whereas six typical maskinonge from the same waters 860 to 911 mm. in length were 9 to 14 + years of age.

Four of the seven peculiar maskinonge reported by Seaborn (6, p. 237) were probably pike–maskinonge hybrids as indicated by the barred pattern and the complete scaling of the cheeks.

Acknowledgments

PLATE I

FIG. 4. *Typical form, 28⅛ in. long, showing pattern of dark vertical bars.* FIG. 5. *Typical form, 34¹¹⁄₁₆ in. long, showing remains of dark vertical bars.* FIG. 6. *Typical form, 43⅛ in. long, showing only traces of dark vertical bars.* FIG. 7. *'True tiger' (amentus) form, 38²⁄₁₆ in. long, showing persistence of dark vertical bars in larger specimens.*

References

1. DYMOND, J. R. The coregonine fishes of northwestern Canada. Trans. Roy. Can. Inst. 24 : 171-231. 1943.
2. EDDY, S. Do muskellunge and pickerel interbreed? Prog. Fish-Cult. No. 48. 1940.
3. EDDY, S. and SURBER, T. Northern fishes. Univ. Minn. Press, Minneapolis. 1943.
4. GODFREY, J., JR. Muskies unlimited. Outdoors. Oct. 1945.
5. HUBBS, C. L. and HUBBS, L. C. The increased growth, predominant maleness and apparent infertility of hybrid sunfishes. Papers Mich. Acad. Sci. 17 : 613-641. 1933.
6. SEABORN, E. Variations in the maskinonge of the Sauble River, Ontario. Copeia, 1937. No. 4. 1937.

THE ANATOMY OF *MONODONTOMERUS DENTIPES* BOH., AN ENTOMOPHAGOUS CHALCID[1]

By Gordon E. Bucher[2]

Abstract

The anatomy of the adult of *Monodontomerus dentipes* Boh., a chalcid parasite of sawfly cocoons in Europe and America, is described. An attempt is made to homologize the structures of this highly modified insect with those known in more generalized insects, in the hope of clarifying some questions of chalcid morphology which has been generally neglected by entomologists. The nomenclature employed is of a kind generally acceptable to students of morphology, an effort being made to eliminate the use of terms specific to a limited group of insects. The anatomy of *Monodontomerus* is not widely different from that of phytophagous chalcids described by other authors.

Introduction

This anatomical study of *Monodontomerus dentipes* Boheman was suggested by work at the Dominion Parasite Laboratory, Belleville, Ont., during the summers of 1936-38. This insect was imported, along with many others, in an attempt to combat the spruce sawfly, *Gilpinia hercyniae* Htg., by means of parasites. The chalcidoid group, as a whole, has been neglected morphologically, and it was felt that *Monodontomerus dentipes* would illustrate the anatomy of an entomophagous chalcid.

Considering the enormous numbers of chalcids and their importance as factors in biological control, comparatively little research has been done on their anatomy or morphology. Bugnion (1) published a long paper on *Encyrtus fuscicollis* but chiefly dealt with the postembryonic development and only touched on the adult anatomy. Imms (5, 6), in his papers on the chalcid parasites of Coccidae, investigated the chalcid ovipositor. The most detailed observations have been made by James (7) on *Harmolita graminicola*, a phytophagous chalcid. James apparently ignored the morphological work of Snodgrass, particularly that on the thorax of the Hymenoptera (10), so that some of James's conclusions with regard to the chalcid thorax are in need of revision. The most recent work on the anatomy of the chalcids has been published by Hanna (3, 4).

The genus *Monodontomerus* (Westwood 1833) belongs to the chalcidoid family Callimomidae, whose members are largely parasitic. It contains a number of species of which the best known is *M. aereus* Walker, which was imported into the United States to combat gypsy moth and browntail moth (9).

Monodontomerus dentipes Boh. is widely distributed in Europe as a parasite of several sawflies, particularly *Diprion similis* Htg. In Canada it has not

become established on the spruce sawfly but is present locally as a parasite of the introduced pine sawfly *Diprion similis*. It has also been recovered from cocoons of *Lophyrus* sp. on pine in New York State (8).

Material and Technique

Cocoons of *Diprion similis*, containing prepupae of *Monodontomerus dentipes*, were obtained from the Dominion Parasite Laboratory at Belleville and by collections at the Sheridan Nurseries at Oakville, Ont. By storing these in a cool place and incubating them as required, a supply of fresh adults was available at all times.

The usual methods of serial sections and dissections were employed in the study of the anatomy. Dissections were made in a variety of liquids, the most useful being Ringer's solution, 70% alcohol, and a mixture of alcohol and glycerin. A 0.5% solution of acid fuchsin sometimes assisted in distinguishing structures. The skeleton was studied with the aid of specimens cleaned by a 10% solution of sodium hydroxide, bleached by a mixture of potassium chlorate crystals, hydrochloric acid, and alcohol, and stained with acid fuchsin.

A number of techniques were used to obtain serial sections. The most satisfactory sections were obtained by using celloidin as an embedding agent. The best fixation was secured with Lebrun's modification of Carnoy's fixative. Ehrlich's haematoxylin and eosin and iron haematoxylin and erythrosin were the best stains.

General Anatomy

Monodontomerus dentipes is a large insect when compared to most of the other chalcids. Since a number of individuals may mature in one cocoon of the host, the parasite is subject to considerable variation in size, depending on the size of the host and the number of emerging parasites. The following measurements may be considered as only roughly modal. The female is 3.4 mm. long from the front of the head to the tip of the abdomen, but measures 4.1 mm. to the tip of the exserted ovipositor. The female thorax is 0.7 mm. broad and 0.8 mm. deep, while the abdomen is 0.7 mm. broad and slightly deeper. The male is 2.7 mm. long, its thorax 0.6 mm. wide and 0.7 mm. deep, its abdomen 0.5 mm. wide and of about the same depth. Except for variations in size and differences in the genitalia, terminal abdominal segments and related parts, the male is similar to the female. Thus all drawings are of female parts unless otherwise stated.

The cuticle is thick, tough, elastic, and dark green in color with a metallic sheen in certain lights. Its smooth, shiny surface is marred by a fine reticulation forming geometrical figures of inconstant shape and size (Fig. 47). Fine setae are scattered over the surface but occur most frequently at the corners of these figures. Surfaces which are subject to friction have the reticulation suppressed and the setae very fine or absent, as for example the occiput, the mesal surface of the legs, the ventrolateral surfaces of the thorax, and segments

three and four of the abdomen where the hind legs rub. Three sclerites have no reticulation or setae and thus stand out by comparison. These are the mesepimera and the posterior division of the scutellum. Setae are best developed on the dorsolateral portions of the propodeum but are nowhere visible to the naked eye.

The insect is dark green in color except for the red compound eyes, parts of the legs, and antennae. The tibia are amber; the tarsi are also amber but lighter in color except for the fifth tarsal segments and pretarsi, which are fuscous. Distally the profemora shade from green to amber, but the other femora and all coxae are dark green. The trochanters shade from dark green to medium brown but retain the metallic green luster throughout. The flagella of the antennae are black but in certain lights show a metallic green sheen. The basal segments of the antennae are green.

The Head

THE EXOSKELETON

The head, viewed from the front, is subtriangular in shape, the dorsum being at the base, the mouth parts at the apex, and the compound eyes occupying the sides of the triangle. It is convex in front and concave behind, where the prothorax fits into the occipital region. The head is strictly hypognathous, but upon death, muscular contraction causes it to assume an opisthognathous position, so that the mouth parts are posterior in position and the head itself hides the prosternum (Fig. 24). The head is as broad as any other part of the body, being about 0.9 mm. wide in the female and 0.8 mm. wide in the male.

The Frons

Since the epicranial and frontal sutures are absent in *Monodontomerus*, the areas of the head are not sharply marked out. The frons (Figs. 1, 2, 3, FR) occupies the region between the antennae, stretching from the clypeus to about the median ocellus (MO). It is usually considered to include the median ocellus and the area around it. Since the antennal sockets (ASO) are close together, the frons is narrow. Dorsal to the antennal sockets, the frons is excavated on each side, to accommodate the scapes of the antennae when in repose (FEX).

The Parietals

The regions lateral to the frons are the parietals (Fig. 3, PRTL). They bear the compound eyes, the lateral ocelli (LO), and the antennae. Since the frons is narrow, the parietal regions are large in *Monodontomerus*. The compound eyes (E) are huge organs, occupying practically the whole lateral surfaces of the head. The cuticula is slightly ridged at the border of the compound eyes with the parietal region but a distinct ocular sclerite is not present. Fine short setae are scattered over the surface. The facets of the eye are small and numerous. The lateral ocelli are similar to the median ocellus. All are ellipsoidal but the orientation is different (Fig. 1).

The Clypeus

The anterior tentorial pits (AT) can be seen on each side, a short distance from the middle line near the anteroventral apex of the head (Fig. 3). Joining them is an indistinct groove, which is all that remains of the epistomal suture (ES). Thus the clypeus (CLP) is bounded by the anterior tentorial pits, the suppressed epistomal groove, and the ventral edge of the head capsule. It is quite small in this form. Extending dorsally from the anterior tentorial pits on each side are other ill-defined grooves, which partially mark off a region somewhat more convex than the rest of the anterior surface of the head, and behind which lies the pharynx.

The Genae

On account of the large size of the compound eyes, the genae (Fig. 2, GE) are reduced in size. They lie in a lateral position, between the compound eyes and the ventral edge of the head capsule. A vertical subocular suture (SOS) divides the area into two halves.

The Vertex

The vertex (Figs. 1, 5, VX) is the area on the dorsum of the head. It has one definite posterior boundary, the occipital suture. It is considered as lying behind the ocelli and between the compound eyes. Since the head is wide in *Monodontomerus*, the vertex is correspondingly large.

The Occipital Arch

From a posterior view the occipital arch appears as a concave horseshoelike sclerite, bounded by the occipital and postoccipital sutures (Figs. 5, 6, OCS, POCS). The dorsal part is known as the occiput (OC), while the lateral parts, lying posterior to the genae, are the postgenae (PGE). The occipital and postoccipital sutures are both well developed. The occipital suture separates the occipital arch from the vertex dorsally and from the parietal and genal areas laterally.

The Postocciput and Foramen Magnum

The postoccipital suture separates the occipital arch from the postocciput (POC), the area surrounding the foramen magnum or occipital foramen (FOR).

The occipital foramen is small and, just inside the head, is crossed by the body of the tentorium, which divides it into a dorsal larger aperture, for entrance of the oesophagus, and a ventral smaller neural aperture. At this division, the sides of the foramen are thickened and are inflected posteriorly to articulate with the proepisterna. These are the occipital condyles (OCC).

The Gula

"In the generalized insects . . . . there is no ventral sclerotization of the head wall between the foramen magnum and the base of the labium, the submentum being directly continuous with the neck membrane between its lateral attachments to the cranial margins just behind the posterior tentorial pits." (Snodgrass (11)). In *Monodontomerus* the labium is not attached to

the cranium directly but articulates with the maxillae, which in turn articulate with the head near the end of the posterior tentorial arms. The area between this articulation and the foramen magnum is a definite sclerite (Fig. 5, GU), and has been called the gula (7). The morphology of this part is in doubt. Snodgrass considers the gula as a typical coleopterous structure which possibly occurs in some other prognathous insects and which is continuous with the mentum. In this region, certain Hymenoptera have a sclerotized plate called the hypostomal bridge (11), which is formed by mesal extension and union of the postgenae. This has apparently not happened here, since the postoccipital sutures are still in place and divide this area from the postgenae. It seems best to retain the name gula pending further investigation, even though it is not in contact with the submentum. The elongation of the posterior tentorial pits on either side of the gula suggests a growth downward of this region from the lip of the foramen. This would be more like the condition in Coleoptera.

The Subgenae

A suture from the posterior tentorial pits, extending ventrally and laterally on each side to the edge of the cranium above the mandible, cuts off a narrow sclerite around the posterior portion of the gnathal cavity. Its morphology is unknown but it seems to correspond most closely to the subgenal suture of generalized insects (Figs. 5, 6, SGS). The sclerites defined by this suture may be called subgenae (SGE).

THE ENDOSKELETON

The occipital, postoccipital, and subgenal sutures occur internally as inflections of the cuticle forming minute ridges which doubtless contribute to the support of the exoskeleton.

Along the whole length of the anteromesal border of the compound eyes the cuticula is inflected, forming a deep internal vertical flap or apodeme which separates the ommatidia from the other organs of the head (Fig. 104).

The Tentorium

The tentorium forms the most important endoskeletal support for the head. It consists of a pair of anterior arms and a pair of posterior arms which join together to form the body of the tentorium (Figs. 4, 6). The dorsal arms, which are found in some insects, are absent here.

The anterior tentorial arms (ATA) arise by cuticular invaginations from the anterior tentorial pits (AT) on each side of the clypeus. They extend dorsally and posteriorly forming an arch. Laterally they are expanded into small lamellae (TL), but they lack the median expansion found in some other chalcids (4). The lamellae assist in supporting the supraoesophageal ganglion.

The posterior arms (PTA) arise from the posterior tentorial pits (PT). These are elongate pits, the ventral ends of which lie in the postoccipital sutures but which dorsally invade the postocciput. They show poorly in a fresh specimen but can be found in one cleaned, bleached, and stained. The posterior tentorial arms are much shorter than the anterior arms. They are

directed dorsally and anteriorly to meet the anterior arms. The combined arms on each side continue dorsally and meet finally in a horizontal bar, dividing the foramen magnum into two parts. This bar is the body of the tentorium or corporotentorium (CT). The posterior arms are expanded ventrally towards the articulations of the cardines with the edge of the head capsule.

From the posterior tentorial pits, an internal ridge (ITR) extends to the border of the foramen magnum and supports it. It is revealed externally by a darkening of the chitin. The whole border of the foramen magnum is somewhat thickened for support.

The Head Appendages

The Mouth Parts

The mouth parts consist of the typical parts found in mandibulate insects, and are adapted for biting, rasping, and licking.

The Labrum

The labrum (Figs. 3, 94, LB) is a minute horizontal plate hinging on the clypeus and bearing about five setae. Rarely is it visible from a frontal view; its relations are best seen in sagittal sections (Fig. 106).

The Epipharynx

The epipharynx (Fig. 94, EPH) is a small fold attached to the labrum and forming the roof of the mouth. It is also best seen in sagittal sections (Fig. 106).

The Mandibles

The mandibles are well developed, subtriangular in shape and equal in size (Figs. 2, 3, 6, 13, 14, MD). They are convex anteriorly. From this view they hide most of the other mouth parts (Fig. 3). The opposing margins of each mandible bear two pointed teeth, an apical and a subapical, and, above these, a molar projection. On the posterior surface are two conspicuous skeletal rods which seem to be characteristic features of the chalcidoid mandible; each arises near the apex of a mandibular tooth and ends freely in a club (Figs. 13, 14). Suggestions of these are also seen from the front. The mandibles are articulated with the head in two places. The anterior articulation consists of a condyle on the anterior edge of the gnathal cavity between the subocular suture and the clypeus, which fits into a socket (A) on the anterolateral corner of the mandible. Posteriorly a condyle (C) of the mandible articulates with a depression in the subgena. This articulation is not so well developed as the anterior one. Therefore, while the primary movement of the mandibles is in a transverse plane, a certain flexibility of movement is attained.

The Maxillae

The maxillae (Figs. 2, 5, 7, 8, 9) possess all the parts of the typical maxillary appendages in insects. The basal portion or cardo (CD) of each maxilla

articulates by a shallow socket (A) with a condyle at the lip of the gula (Fig. 5). The stipes (ST) is attached to the distal margin of the cardo. The palpifer of the stipes cannot be distinguished, the four-jointed maxillary palp being borne directly at the distal end of the lateral margin. The galea and lacinia of the maxilla are reduced and somewhat modified from the typical form. The lacinia (LC) articulates with the distal portion of the stipes and ₊assumes a "U" shape, the open part of the "U" being anterior. Thus, components of the lacinia are both lateral and medial. The galea is borne on the lateral side of the lacinia and is attached to the posterior and distal edges of the lacinia. The cuticle, along the line of attachment of the galea to the lacinia, is lighter in color but only slightly more flexible and thus the two parts tend to move and function as a whole. The median portion of the lacinia has a membranous connection with the galea on the medial surface.

The Labium

The labium (Figs. 2, 3, 5, 10, 11, 12) is situated between the maxillae. It consists essentially of a strongly convex plate, the mentum (MT), bearing terminally an unpaired ligula and a pair of three-jointed palps. Proximally the mentum articulates with an ovoid submentum (Fig. 5, SMT), which is only a slight sclerotization of the membrane. Laterally the mentum articulates with the basal part of the lacinia (A). The fleshy ligula (LIG) is borne distally by the mentum. It is a rasping and licking organ. The posterior face is flat but the anterior surface is strongly convex and bears eight or nine serrations which are more rigid than the rest of the organ. The ligula is supported laterally and posteriorly by a pair of sclerites (LSC).

The inner surface of the mentum is occupied by a small lobe, the hypopharynx (Figs. 12, 106, HPH), which is best seen in section as it usually tears away on dissection. Between the hypopharynx and the ligula is the opening of the salivary gland. On either side of the hypopharynx is a skeletal rod (HSC), which lends support to the hypopharynx and labium since the lateral surface of the mentum is only lightly sclerotized.

THE ANTENNAE

The antennae of the male and female are similar except for the difference in size. The scape (Figs. 3, 45, 46, SCP) is the longest segment. It articulates with the head, not directly as is usual in insects, but by a basal segment (BS) as in some other chalcids (6). This appears to be a further modification of the articulating bulb at the base of the scape in many insects. In *Monodontomerus* the bulb has been separated completely and is present as a basal segment.

The antennal sockets (Fig. 3, ASO) are situated close together near the center of the frontal surface of the head. The rim of each socket is strengthened by an internal submarginal ridge appearing on the exterior as an antennal suture (AS). A pivotlike process, the antennifer (AN), on the ventrolateral rim of the antennal socket articulates with the basal segment of the antenna.

The pedicel (Figs. 45, 46, PDC) in *Monodontomerus* is short.   Investigation for a possible organ of Johnston was not attempted.

The flagellum, considered as that part of the antenna distal to the pedicel, consists of nine segments.   The first or ring segment (RS) is narrow and is found in most chalcids.   The remaining segments of the flagellum are subequal with the exception of the terminal one.   It is club-shaped, larger than the others, and shows evidence of being compound, since it is divided into three annuli by two sutures.

A mesal extension of the cuticle at the ends of each segment of the funicle reduces the intersegmental channel greatly.   From the lip of the anterior foramen thus formed there extends a narrow flange, which articulates with the lip of the posterior foramen of the next segment (Fig. 15, A).

The basal segments of the antenna have scattered setae but the segments of the flagellum beyond the ring segment are covered by numerous setae. They are further characterized by the possession of elongated sensory pits (Fig. 15, SP), which show up light against the black cuticle.   These pits are typically arranged in two rows surrounding each segment.

The Prothorax

The prothorax is considerably reduced and the pronotum is only loosely connected to the other parts of the segment, thus leaving the prosternum and propleura to act as a suspensorium for the front legs.

The Protergum

The protergum (Figs. 16, 17, 18, 20, 22, 23, 24, 25, 26, PRT) is similar in shape to a horse-collar, being produced ventrally to hide the remainder of the prothorax.   Posteriorly it covers a considerable portion of the mesothorax and at its dorsolateral corners the cuticle is inflected forming a pair of flanges (PMA) for articulation with the mesonotum.   The mesothoracic spiracle opens just below this flange.   Anteriorly the edge of the pronotum flares in the middle line and fits against the occiput just above the foramen magnum, thus forming a secondary articulation with the head (PHA).   Posteriorly the sides of the protergum are connected by a membranous band which overlies the mesophragma.

The Propleura

The paired episterna (Figs. 16, 18, 19, 20, 21, 22, 26, EPS) comprise the whole propleura of *Monodontomerus*.   They fuse ventrally with the prosternum and curve upwards to a lateral and dorsal position beneath the pronotum.   Anteriorly each sclerite bears a heavily sclerotized projection (CV) for articulation with the occipital condyles of the head.   These projections probably represent the cervical sclerites which have been fused with the propleura.

THE PROSTERNUM

The prosternum (Figs. 16, 18, 19, 20, 22, 24, 26, STN) is roughly diamond-shaped. At the posterior edge it is reflected upwards between the coxae. James (7) has given the convenient name of reduplication to this inflected part (Figs. 19, 20, 22, PSR).

The arms of the profurca (Figs. 19, 20, 22, AFU) are well developed, arising from the short, low manubrium of the furca (MFU) and the reduplication of the prosternum without an evident pit. They curve outwards to meet the dorsal inflected portions of the episterna, with which they fuse.

The Mesothorax

The mesothorax, being the chief wing-bearing segment, is the largest segment of the thorax. It is highly developed and modified but nevertheless retains a more primitive structure than the other thoracic segments, since most of the sclerites, present in generalized insects, can be identified.

THE MESOTERGUM

The mesotergum is a large compound plate, arched longitudinally and extremely convex, extending far down on each side. It is marked off into surface areas by a number of external sutures, which, with some trouble, can be homologized with those of a typical alinotum (Figs. 23, 25, 27, 28, 29, 33).

The primary suture of the alinotum is a ⌒-shaped suture, lying in a posterior position with its apex directed forward, and dividing the notum into an anterior scutum (SCT) and a posterior scutellum (SCL). Internally there is a strong corresponding ridge which, in *Monodontomerus*, is prolonged into a short flap or apodeme (Figs. 29, 33, VR). This primary suture is called the scutoscutellar suture (VS).

A transverse transscutal suture (TSS) cuts completely through the scutum, setting off two posterolateral areas of the latter from the major scutal area. The parts of the alinotum separated by this suture are frequently called scutum and scutellum and the suture known as the scutoscutellar suture, particularly by students of Hymenoptera. It is evident that this suture does not correspond to the scutoscutellar suture, since the latter is also present. It may also be pointed out, that, typically, the scutellum is a plate cut off completely from the wing articulations, while the transscutal suture divides the scutum into an area associated with the anterior notal wing process and one associated with the posterior notal wing process. Since the posterolateral areas of the scutum, defined by the transscutal suture, seem to be without an acceptable name, they may be called postscutum (PSCT), for convenience. They correspond to the prescutellum of Hanna (4).

The anterior area of the scutum is divided into three parts by two sutures extending forwards and outwards from the transscutal suture. These are known as lateral sutures or parapsidal furrows (PF) and separate the dorsolateral plates or parapsides (PAR) from the median scutum. These sutures

are apparently identical with the convergent sutures or notaulices of other forms. Under the mistaken idea that the parapsidal furrows were discontinuous parts of the transverse prescutal suture turned posteriorly, some morphologists named the area between them prescutum. This is erroneous since the parapsidal furrows and prescutal suture are both present in some Tenthridinidae. There is no transverse prescutal suture or corresponding prescutal plate in *Monodontomerus*.

The parts of the alinotum may be summarized as follows. A scutoscutellar suture separates the scutellum from the scutum. The scutum is divided by a transscutal suture into an anterior portion and a posterolateral portion, the postscutum. The anterior portion is further subdivided into a single median scutum and lateral parapsides by a pair of parapsidal furrows.

The phragmanotum or postnotum (Figs. 27, 33, PN) is the other major division of the typical pterothorax. In *Monodontomerus* it is completely hidden. The parts of the alinotum may now be considered in more detail.

The Scutum

The scutum (Figs. 23, 25, 27, 28, 29) is a large median plate bearing in front the prephragma (Figs. 27, 29, 31, 1PH). The line of division probably represents the antecostal suture (Fig. 27, ACS) but the acrotergite before the antecostal suture cannot be recognized.

The parapsidal furrows, separating the scutum from the parapsides, are well developed. Internally they form wellmarked ridges (Fig. 29, PR) which are produced into flanges particularly where they meet the prephragma. At this point the scutum bulges somewhat, forming the chief surface (MPA) for articulation with the pronotum.

The Parapsides

From their dorsal connection with the scutum the parapsides (Figs. 23, 25, 27, 28, 29, PAR) curve outwards and downwards to meet the pleura, and thus form a prealar bridge (Figs. 23, 27). Along this lower margin the cuticle is inflected forming a flange (Fig. 29, PFL). The parapsides are cut off from the flange by a submarginal external ridge (PSR). Each parapsis bears a lateral backwardly directed plate which may be called the anterior alar plate (AAP). At the line of fusion of this plate with the parapsis, the latter is prolonged backwards into a short rim (PER). The anterior alar plate, which begins in a vertical plane, flares laterally at the posteroventral corner, forming the anterior notal wing process (ANP) for articulation with the first axillary sclerite.

The tegulae (Figs. 23, 24, 25, 27, TEG) are convex sclerites embracing the bases of the front wings and loosely articulated along the lower edge of the anterior alar plate.

The Postscutum

The postscutum is divided into two dorsolateral areas by the forwardly projecting scutoscutellar suture. Each postscutal plate (Figs. 23, 25, 27, 28, 29, PSCT) is partially divided into two regions by a short suture (PSS),

extending part way into the plate from the scutoscutellar suture. Internally a small ridge corresponds to this suture. The contour of the plate is complicated. It roughly takes the form of an "S" sloping downwards and backwards from the transscutal groove and sloping downwards, outwards, and forwards from the scutoscutellar suture, the suture of the postscutum lying in the excavation so formed. Each postscutal plate bears a posterior alar plate (PAP) corresponding to the anterior alar plate of the parapsis. At the junction of the two, the postscutal plate bears a heavy flange (SFL) which, however, does not reach the notal margin. The posteroventral angle of the alar plate flares laterally, forming a large projecting spine, the posterior notal wing process (PNP), at the base of which the third axillary sclerite articulates.

As mentioned above, the postscutum is separated from the scutum and parapsides by a transscutal suture. In most insects this suture is defined internally by a strong ridge lending support to the mesonotum. In *Monodontomerus* and certain other chalcids (7) this suture forms a zone of weakness and movement, the two parts of the scutum being joined by membranous or semimembranous integument. This probably led James erroneously to name this the scutoscutellar suture. Internally there is no strong transverse ridge, but the postscutal plate on each side bears a weak apodeme (Figs. 28, 29, SPS) directed forward to lie in a horizontal position beneath the corresponding parapsis. Since their resemblance to true phragmata is strong, these may be termed pseudophragmata.

The Scutellum

The scutellum (Figs. 23, 25, 27, 28, 29, 33, SCL) is a large plate somewhat more convex than the remainder of the mesonotum, occupying a posterior median position, and being defined from the scutum by the scutoscutellar suture which internally forms a well-marked ridge. The posterior third of the scutellum is set off from the rest by a transscutellar suture (TSCS) which internally forms a very fine low ridge. This posterior plate is characterized by the absence of reticulation and setae and thus is very shiny. The posterior part of the scutellum is traversed by a wide submarginal groove in which the cuticle is thrown into folds, forming a row of wide pits separated by narrow elevations. The marginal part of the plate is a production of the scutellum overhanging the metanotum.

The Postnotum

The postnotum (Figs. 27, 33, PN) of *Monodontomerus* is a narrow flexible plate, weakly attached to the scutellum in front and the metanotum behind, and entirely hidden from view by these structures. Anteriorly it bears an apodeme, lying horizontally beneath the scutellum, which may be termed a pseudophragma (PPS). Posteriorly the postnotum bears the large postphragma (2PH), the posterior attachment of the dorsal longitudinal muscles. The phragma is highly convex with deep lateral areas. It extends backwards and downwards to underlie the metanotum and propodeum and almost reaches the articulation of the petiole with the latter. On each side the postnotum

articulates with the flexible postalar bridge, which extends downwards to connect with the mesepimeron. The postalar bridge (Figs. 23, 27, 28, AB) consists of a dorsal portion of definite shape and considerable rigidity and a ventral portion rather variably marked and comparatively flexible, the line of division indicating a zone of movement.

THE MESOPECTUS

The mesosternum and mesopleura are combined into a compound structure, to which Snodgrass has given the name pectus. The mesopectus in *Mono-dontomerus* is a large, deep, boat-shaped structure, divided by a transverse suture into an anterior area or prepectus (PRP) and a posterior area, which is further subdivided by a median ventral longitudinal groove into two identical halves. Each half is divided by an oblique pleural suture (PS) into an upper epimeron (EPM) and a ventrolateral sternoepisternal plate.

The Prepectus

The prepectus (Figs. 23, 24, 26, 27, 30, 31, 32, PRP) is a narrow unpaired sclerite set off from the mesopectus by a flexible suture. Dorsolaterally it expands and its dorsal margin on each side articulates with the parapsis and forms the prealar bridge. Internally it bears anterior, posterior, and dorsal submarginal low ridges (Figs. 19, 30, IRP). There are no corresponding external sutures but the line of each ridge is indicated by the greater density of the skeleton. These lines, along with an oblique folding of the cuticle, mark off a visible external triangle on the lateral surface (Fig. 23, TP).

The Mesosternum

The mesosternum (Figs. 24, 26, 30, 31, STN″) is a poorly defined area in *Monodontomerus*. A median longitudinal sternal groove divides it into two halves. This sternal groove marks the inflection of the sternum to form the furcal base. Since the base of the furca is large, almost the whole of the sternum may have been so inflected and the remainder may be represented here merely by the suture. This, however, is pure conjecture and until further evidence has been presented, it is best to consider the sternum as that area occupying a ventral position in the mesopectus. Posteriorly the sternum is reflected dorsally between the bases of the coxae to form a posterior reduplication (MSR) as mentioned above for the prothorax. At the base of the reduplication a definite pit appears, marking the posterior inflection of the sternal apophyses (FP). A pair of spines (Figs. 28, 30, MSRA), borne by the reduplication of the sternum, form a secondary articulation for the mesocoxae.

The Episternum

The mesosternum is not separated from the episternum, which, therefore, must be considered as the lateral part of the combined plate. The episternum (Figs. 23, 27, 32, EPS) is separated by an oblique suture (PS) from the epimeron. This suture stretches from the coxal articulation (A) towards the pleural wing process (PWP), but, before reaching the latter, it bends forwards,

completely separating the episternum from any part in the formation of the wing process. It is beyond the scope of this paper to determine whether this is, in whole or in part, homologous with the true pleural sutures of more generalized insects.

The Epimeron

The epimeron (Figs. 23, 24, 25, 26, 27, 30, 31, 32, EPM), at its anterodorsal angle, is prolonged into a low extended pleural wing process (PWP). The remaining dorsal edge is inflected and produced into a spine for articulation with the postalar bridge (Figs 27, 32, EP). The contour of the epimeron is complex in the dorsal region; the reader is referred to Fig. 23, where shading has been used to indicate depression. The epimeron is characteristic in having no reticulation or setae. Its shiny surface, however, is marred by a dimple (D) about the middle of the plate.

THE ENDOSKELETON

Internally the mesopectus is variously strengthened. Its anterior margin is inflected forming an apodeme. The dorsal margin is similarly inflected, forming the spine (EP) for articulation with the postalar bridge. The horizontal part of the pleural suture bears an internal ridge (Figs. 27, 28, 30, 32, IPR) which joins with the spine. The oblique portion of the pleural suture is without an internal ridge. The pleural wing process is also strengthened by a ridge (IWR), which in turn unites with this complex.

The Mesofurca

The endosternum of the mesothorax arises by the inflection of the sternum along the middle line, forming a longitudinal vertical plate sometimes called the manubrium or shaft (Figs. 19, 30, 32, MFU). The manubrium forks dorsally, giving off a lateral arm (AFU) at each side, so that the whole structure, the furca, roughly takes the shape of a "Y" and supports the mesothoracic ganglion. Anteriorly the furcal arms are joined by an arch (FA), which overlies the ganglion. Each arm proceeds forward and upwards towards the epimeral spine, to which it is attached by a tendon. In generalized insects the arms of the furca articulate with the pleural process, a projection of the pleural ridge. This suggests that in *Monodontomerus* the epimeral spine represents some part of the pleural ridge. Anatomical evidence reveals it as merely an infolding of the edge of the epimeron. Since, as has been mentioned above, the pleural ridge joins the spine, there are, doubtless, some pleural elements incorporated in it and thus it is not surprising that the furcal arm unites with it.

The Metathorax and Propodeum

The metathorax is greatly reduced in *Monodontomerus*, consisting of a bandlike metanotum and a metapectus which includes both sternal and episternal elements. As in other clistogastrous Hymenoptera, the first abdominal segment has been incorporated with part of the metathorax to form the propodeum which will, therefore, be considered along with the metathorax.

THE METANOTUM

The metanotum (Figs. 23, 25, 38, 39, 40, 41, MN) is a narrow transverse sclerite, lying between the scutellum and the propodeum, and being loosely articulated to the latter and to the concealed postnotum in front. At the sides, it dips abruptly forming a vertical alar plate which bears the pointed anterior notal wing process (ANP) and the rounded posterior notal wing process (PNP). A heavy ridge separates the metanotum from its alar projection. The cuticula of the metanotum is thrown into external folds of fairly constant character marking off wide shallow trenches. The metanotum bears no phragmata.

The metanotum articulates with the postalar bridge on each side by means of a small sclerite (Figs. 27, 28, 68, X), which meets the metanotum in the region of the anterior notal wing process.

THE METAPECTUS

The metapectus (Figs. 23, 26, 34, 39, 40, 41) consists of the combined sternal and pleural regions of the metathorax.

The Episternum

The episternum (EPS) may be considered as the triangular portion of the metapectus visible from the side. Dorsally it is separated from the propodeum by a longitudinal pleural suture (PS), which stretches from the coxal articulation to the pleural wing process (PWP). Internally this suture forms the pleural ridge (IPR) with which the metafurcal arms join. Thus, it seems to be the true pleural suture; and, therefore, the plate below must be episternum, while the epimeron is fused above with the propodeum.

The Metasternum

The metasternum (STN''') consists of a narrow sclerite in front of the hind coxae. It is continuous at the sides with the episterna. Posteriorly it curves upwards between the hind coxae as the reduplication of the metasternum (MTR), and meets the propodeum just below the lower lip of the propodeal foramen.

THE PROPODEUM

The propodeum (Figs. 23, 25, 38, 39, 40, 41, PD) is a large dorsal sclerite, consisting of the first abdominal segment fused with part of the metathorax. The author believes that both the metapostnotum and metepimera are included in the propodeum of *Monodontomerus*. At the sides it is produced forward to form the ·pleural wing process (PWP). Posteriorly there is a large foramen (Figs. 40, 41, FOP) in the propodeum into which the petiole fits. It is thought that, being an abdominal segment, the propodeum is composed of a dorsal and a ventral plate, the latter being represented by the lower rim of the foramen. There is no well marked suture dividing the reduplication of the sternum from the propodeum, but the manubrium of the endoskeleton ends some distance from this foramen and it probably marks the limit of the metasternum. The integument of the propodeum is thrown

into external folds of fairly constant character. Those near the coxal articulations are more subject to variations. The whole metathorax of *Monodontomerus* may be characterized by the abundance of such folds. In the figures these folds have been represented by heavy lines.

THE ENDOSKELETON

The endoskeleton of the metathorax consists of a pair of diverging furcal arms (Figs. 19, 39, 40, 41, AFU), arising near the anterior edge of the sternum without leaving a noticeable pit. Each arm expands into a plate which fuses at the sides with the large pleural ridge. The whole anterior edge of the metapectus (INP) is inflected for a considerable distance. Dorsally this inflection joins with the pleural ridge. Behind the pleural arms a low manubrium (MFU) arises from the sternum and its reduplication. Externally no groove is apparent along this line though a greater density of the chitin shows externally as a line.

The Legs

The legs are, in general, of the cursorial type, although the metathoracic legs are greatly developed in connection with the habit of jumping into the air prior to flight. Detailed description of the legs seems unnecessary and the reader is referred to the figures (Figs. 42, 43, 44, 48, 49, 50). The tarsi (TAR) are five-jointed and bear a terminal pretarsus (PTAR). Ventrally they have large movable setae. The tibiae of the prothoracic and mesothoracic legs bear a single spur or calcar (CC) while the metathoracic tibia (TB) bears two such spurs. The latter also possesses a number of short spines dorsally and terminally. The mesofemur (FM) is divided proximally by a definite suture at which there is no movement. The sclerite thus set off is characteristic of many parasitic Hymenoptera and is called the second trochanter (2TR). The profemur shows similar incomplete division but no evidence of this can be detected in the metafemur. The metafemur bears a distal toothlike projection (DF). Similar but less definite teeth are formed on the procoxa and metacoxa by a flaring of the integument. The coxae (CX) are comparatively large and cover most of the thorax ventrally (Fig. 24).

The Articulations

The articulations of the legs follow the general insect form. The procoxa (Figs. 21, 51) articulates with the inner angle of the episternum without the aid of a specialized condylar apparatus. The meso- and metacoxal articulations (Figs. 52, 53) are monocondylic, a process developed at the end of the pleural suture articulating with a shallow socket on the coxa. Thus all three coxae, having a single articulation with the pleuron, are not limited to uniplanar movement. They may be observed to move in a rotary manner. The coxotrochanteral joint (Figs. 54, 55, 56) in all three legs is dicondylic though one condyle is poorly developed in the prothoracic leg. There is a certain flexibility throughout at this joint. The trochanterofemoral joint (Figs. 54, 55, 56) of all the legs is without any definite condylar articulations. The movement is very restricted at this joint. The femur and tibia articulate

by a hinge joint or ginglymus allowing movement only in one plane (Figs. 57, 58, 59). The tibiotarsal articulations are monocondylic (Figs. 60, 61, 62, 63).

The Pretarsus

The pretarsus (Figs. 42, 43, 44) is similar in all three legs and corresponds closely to that found in other insects. It consists essentially of a pair of large lateral claws, the ungues (UN), and a median fleshy lobe, the arolium (AR). The claws are bilobed having a long curved upper point and a wider basal lobe. They articulate dorsally with the last tarsomere, which is not differentiated into a distinct unguifer. The arolium is supported dorsally by a flask-shaped orbicula (OR) and ventrally by the calcanea or unguitractor (UNG). An incomplete ringlike sclerite, the camera (CA), supports the arolium in a more terminal position.

The Wings

The wings of *Monodontomerus* (Figs. 65, 66) are similar to those of other chalcids in having reduced venation, and a condensation of the bases of the veins to form scales or sclerites for articulation with the axillary wing sclerites. The front wing is much larger than the hind one, a feature to be expected since the flight muscles are mainly concentrated in the mesothorax. Both wings work together, however, a frenulum (Figs. 64, 66, FL) of the hind wing catching a stout rib (Fig. 65, WR) on the posterior margin of the front wing. The wings are covered with setae or macrotrichia except for the basal areas which are comparatively naked. When not in use the wings are folded flatly over the back and extend slightly beyond the tip of the abdomen.

Burks (2), who has made a study of chalcidoid venation, considers the submarginal vein (Figs. 65, 66, SMV) to be composed of subcosta and radius (SC + R), and the marginal and postmarginal veins (MV, PMV) to be the first radial branch (R_1). The stigmatal vein (STV) is the second radial crossvein R_2. Rows of macrotrichia trace the paths of the obsolete median and cubitus vein (M + CU) and its branches (M, CU'). The hind wing shows only the submarginal vein.

The Wing Articulations

Each wing is attached to the body by a membranous basal area containing a number of small articular sclerites, the pteralia or axillaries. These are very minute in *Monodontomerus*, particularly in the hind wing.

In the forewing the first axillary sclerite (Fig. 67, AX1) is L-shaped. It articulates with the anterior notal wing process of the mesothorax, the edge of the alar plate and the large subcostal scale (SCS) embracing the wing base. The second axillary (Figs. 67, 69, AX2) presents both a dorsal and a ventral sclerotization in the wing base. Its ventral area lies on the pleural wing process and the dorsal edge of the epimeron, while dorsally it articulates with the first axillary and the basal scale of the radius. The third axillary (AX3) articulates with the posterior wing process, the second axillary, and the basal scale representing the condensed anal veins. Ventrally a basalare (BAL)

articulates with the anterior surface of the pleural wing process and with the subcostal scale. The posterior margin of the articular membrane is not thickened into an axillary cord.

In the hind wing the sclerites are similar but much smaller. The first axillary (Figs. 68, 69) articulates with the anterior notal wing process, the edge of the alar plate and the base of the submarginal vein. The second axillary articulates with the pleural wing process, the first axillary and the base of the submarginal vein. The third axillary articulates with the posterior notal wing process, the second axillary, the thickened edge of the wing, probably representing the anal veins, and a small sclerite (M'), which may be the condensed base of the median vein. A basalare joins the submarginal base to the pleural wing process. There is no definite axillary cord.

The Abdomen

The abdomen of *Monodontomerus* (Figs. 70, 71, 72, 73, 74, 75) consists of 10 segments, though much modification has occurred, resulting in a partial or complete disappearance of some of the segments as such. The first abdominal segment has become incorporated into the thorax as the propodeum. The eighth and ninth segments are modified in connection with the development of the external genitalia; they exhibit the most essential difference between the male and female insects. There is such an extraordinary telescoping of the segments that most of the area of the various sclerites is hidden.

The Female Abdomen

The abdomen of *Monodontomerus* appears to be sessile owing to the small development of the petiole, the second abdominal segment (Figs. 35, 36, 37, 70, 72, 73, 2T, 2S). The petiole is a small ringlike segment, embraced by the propodeal foramen in front and articulating with the third segment behind. Internally it bears a transverse chitinous tendon (Fig. 37) which divides it into an upper and a lower portion, probably representing the fused tergum and sternum. The sternum is very slender ventrally but flares backwards at the sides. The walls are dense and rugulose. Through the lumen pass the alimentary canal, the aorta, the nerve cord, and the longitudinal tracheal trunks. The third segment is large, particularly the sternal portion (3S), which is bent into a V-shaped trough along the median line. At the anterior end of the "V" the sternum projects downward forming a distinct lip. The posterior border is without a median indentation so characteristic of many chalcids. At each side of the third abdominal sternum there is a raised portion for articulation with the tergum.

Segments 3, 4, 5, 6, and 7 are all complete. The terga are large and come down the side almost hiding the sterna from a lateral view. Each sternum bears a pair of internal ridges or apodemes for the attachment of intersegmental muscles. The seventh sternum is particularly well developed and has lightly sclerotized lateral portions projecting posteriorly, giving it a Λ-like appearance. The ovipositor issues beyond the seventh sternum.

The eighth segment is represented by a small dorsal tergum and two lateral, lightly chitinized plates projecting forward, the latter being hidden by the terga in front (Figs. 93, 99, 100, 8S). These lateral sclerites are probably the divided eighth sternum. The eighth tergum contains a pair of spiracles (Fig. 99, SP3). The ninth segment is represented by the inner and outer plates of the ovipositor. The inner plates are considered to be the ninth sternum and the outer plates the ninth tergum. This tergum carries a pair of small appendages, bearing five sensory hairs of which one is considerably larger than the rest. James calls these sensory plates (SPL). They have been erroneously called cerci. The inner plates end in a pair of large sensory palps (PAL), covered with setae and enveloping the sting. The female uses these in investigating the host cocoon before oviposition.

The anal papilla (ALP) represents the 10th segment and any segments posteriorly. Dorsally it is hardened, forming a small plate, typically bearing two setae on each side. Often this plate is not symmetrical, owing to an underdevelopment of one side.

THE MALE ABDOMEN

The abdomen of the male (Figs. 71, 74, 75) differs from that of the female principally in size and in the modification of the terminal segments. The eighth segment is complete in the male, being formed of a tergal and a sternal plate. The ninth tergum bears a pair of sensory plates similar to those of the female. The ninth sternum is divided into two halves separated by a narrow longitudinal membrane. The aedeagus (AED) issues from the abdomen beyond the ninth segment. The 10th segment is represented by a small anal papilla. Ventral to the papilla and running forward to join it to the aedeagus sheath (AES) is a narrow lightly sclerotized plate (Fig. 85, VI), which James (7) believes to represent the "valva interna" of Zander. This may be part of the 10th segment.

The Female Reproductive System

The generative organs of the female consist of a pair of ovaries, each possessing an oviduct which unites with its fellow to form a common oviduct and vagina. There are two pairs of accessory glands and a spermatheca or receptaculum seminis, which also possesses a pair of glands.

THE OVARIES

The ovaries (Fig. 83) are long in comparison to the length of the abdomen and thus must be convoluted. They pass backwards from the oviducts, curve upwards in the region of the sixth notum, and pass forwards to the front of the abdomen, then curve downwards and backwards to the ventrolateral regions of the crop, from which they ascend to the dorsolateral regions of the latter. Here the vitellarium changes to germarium and the latter is produced backwards on the dorsolateral surface of the crop.

Each ovary consists of three ovarioles (OVL) which adhere closely, *in situ,* by connective tissue strands. Each ovariole may be divided into two main regions, the germarium and the vitellarium.

The germarium (GER) is the terminal portion of the ovariole and contains undifferentiated cells which are transformed into egg cells and nutritive cells. The germarium adheres to the dorsolateral surface of the crop. No terminal filament could be identified.

The vitellarium (VIT) includes all the remainder of the ovariole and contains eggs in all stages of development. Distally in the vitellarium the eggs (EG) are small, surrounded by the follicular epithelium (FE), and alternate with the nutritive or nurse cells (NC). This polytrophic type of ovariole is characteristic of Hymenoptera in general. Proximally, the nutritive cells have degenerated, a chorion (CH) has been produced by the follicular epithelium, and, at the entrance to the oviduct, there are several mature eggs lying free in the ovarioles. The eggs are banana-shaped, having a short pedicel at one end. The chorion is produced externally into numerous short spines which probably represent the intercellular spaces in the follicular epithelium. Each ovariole of a newly emerged female contains three or four mature or nearly mature eggs, so that about 20 or 25 eggs are ready for oviposition soon after emergence.

THE OVIDUCTS

The three ovarioles (OVL) on each side unite to form an oviduct (OV) which passes forwards and meets its fellow of the opposite side, forming a short median tube, the common oviduct (COV). Two pairs of glands open into the common oviduct. James (7) has named these the primary and secondary accessory glands (1AG and 2AG). The duct of the spermatheca also opens into the common oviduct.

THE SPERMATHECA   ·

The spermatheca or receptaculum seminis (SPM) consists of a short duct, expanded at its end to form a cavity for the storage of sperms and emptying into the common oviduct. Two accessory glands (AGS) are associated with the spermatheca.

THE VAGINA

The common oviduct opens into the vagina (VAG), which passes forward to the base of the sting and overlies the poison sac. The dorsal wall bears a sclerite (Figs. 81, 83, VSC) which becomes very thin at the edges, the latter being produced downwards at the side. This sclerite serves as an insertion for muscles attaching the vagina to the seventh abdominal sternite. The vagina bends downwards around the base of the ovipositor and opens in the middle line, just in front of the base of the seventh sternite to form the female genital opening. This end-portion of the vagina receives the aedeagus during copulation and therefore may be called the bursa copulatrix.

The Female Genital Armature

The genital armature of the female is composed, as in other insects, of three pairs of gonapophyses, developed from the eighth and ninth abdominal segments. In *Monodontomerus* these gonapophyses are greatly specialized to form the ovipositor, an organ for the transference of eggs from the genital aperture to a position within the host cocoon. The ovipositor is the best investigated organ of chalcidoid anatomy. Imms (6), James (7), and Hanna (3) have made studies which agree, both with each other, and essentially with Snodgrass's investigation in the honey bee.

The essential structure of the ovipositor is a long egg-tube formed by the co-operation of paired stylets and stylet sheaths.

The Stylet Sheaths

The stylet sheaths (Figs. 76, 77, 78, 79, 80, 82, STYS) are a pair of long chitinous rods which fuse at the apex and bear toothlike projections, which act as saw-teeth for drilling into the host cocoon (Fig. 79). At its base each sheath expands into a large heavily sclerotized knob, the rotatory process (RP). The paired rotatory processes are connected with one another by a series of transverse chitinous ribs (RPR). The stylet sheaths pass upwards and backwards from the rotatory processes as a pair of diverging arms.

The Stylets

The stylets (Figs. 76, 77, 78, 80, 82, STY) are a pair of long hollow chitinous rods which become pointed and bladelike at the apex. Together with the stylet sheaths they form the essential boring apparatus and egg tube (Fig. 80, ET) of the ovipositor. Laterally each stylet bears a longitudinal groove into which a ridge of the sheath fits. This helps to hold the component parts of the sting in juxtaposition. Anteriorly the stylets diverge from one another but remain in contact with their corresponding sheath. James believes that the poison fluid passes down the stylet canals and enters the wound by one or two fine pores near the apex. Such pores were not seen in *Monodontomerus*.

For the proper functioning of the ovipositor three other components are necessary, the inner plates, the outer plates, and the fulcral plates.

The Inner Plates

The inner plates (Figs. 76, 77, 78, 82, IP) of Imms (6), corresponding to the oblong plates which Snodgrass found in the bee, consist of a pair of long lamellae, diverging and expanding proximally to fuse with the diverging arms of the stylet sheaths. They are produced forward lateral to the rotatory processes as the pivoting sclerite (IPP). Throughout most of its length, each plate bears an inner longitudinal rib (IPR), which contributes to the rigidity. Proximally this rib articulates with the fulcral plate. The plates are joined ventrally by a membranous connection which bulges upwards forming a groove to receive the sting. Near the apex, the outer edges of the plates curl over and join in the mid line forming a short bridge (IPB). Terminally the inner plates bear a pair of sensory palps (PAL) which enclose the sting when at rest.

The Outer Plates

The outer plates of Imms (Figs. 76, 77, 78, 82, OP), corresponding to the quadrate plates of Snodgrass, occupy a lateral position enclosing the inner plates. The dorsal edges are reflected inward, and distally they meet forming the ninth tergum. This tergite bears a pair of sensory appendages with five hairs (SPL). Proximally the outer plates articulate with the fulcral plates. They are strengthened by chitinous ribs (OPR).

The Fulcral Plates

The fulcral plates of Imms (Figs. 76, 78, 82, FP) correspond to the triangular plates of Snodgrass. Each is a narrow sclerite, fused dorsally with the corresponding stylet and articulating with the inner and outer plates.

The various parts of the ovipositor may be homologized with those of generalized insects, there being agreement among the various investigators. The stylets are formed from the gonapophyses of the eighth segment and correspond to the ventral valvulae of orthopterous insects. The fulcral plate is also developed from the eighth sternum and probably corresponds to the first valvifer. The inner gonapophyses of the ninth segment produce the stylet sheaths, which consequently correspond to the inner valvulae of the Orthoptera. The inner plates are developed from the outer gonapophyses of the ninth segment and thus are homologous with the dorsal valvulae or lateral gonapophyses of other insects. The proximal expansions of the inner plates, which fuse with the diverging arms of the stylet sheaths, seem to correspond to the second valvifer. The outer plates, as can readily be seen, are formed from the ninth tergum and distally retain their dorsal connection.

For a discussion of the muscles of the ovipositor and the method of functioning, the reader is referred to James (7), Hanna (3), and Imms (6).

THE POISON APPARATUS

The poison apparatus is well developed and consists of two glands (Fig. 84) which correspond to the acid and alkaline glands of the bee.

The acid gland (ACG) is a long tubular structure lying in the floor of the ovipositor. It has a narrow lumen surrounded by large, columnar, darkly staining cells with prominent nuclei. Anteriorly it opens into a capacious reservoir (ACR), situated between the diverging arms of the sting and beneath the vagina. Its contents coagulate on coming in contact with alcohol. A small duct leads from the reservoir to the base of the sting.

The alkaline gland (ALG) is an elongated structure, overlying the acid gland reservoir and opening into the base of the sting by a fine long duct.

The Male Reproductive System

In the male the reproductive system consists of the paired testes, vasa deferentia, vesiculae seminalis, glandulae mucosae, an unpaired ductus ejaculatorius, and the genital armature.

The Testes

The testes (Fig. 85, TT) are a pair of small sacs, lying above the mesenteron on each side at about the level of the fifth or sixth abdominal tergites. They attain their maximum size in the pupa, since after eclosion of the adult spermatogenesis is largely completed and the testes tend to shrink in size.

The Vasa Deferentia

These are two thick walled tubes (VD), leading from the testes downwards to the vesiculae seminales.

The Vesiculae Seminales

The seminal vesicles (VSM) are morphologically dilations of the walls of the vasa deferentia for the storage of sperm.

The Glandulae Mucosae

These are bean-shaped mucous glands (GM) lying lateral to the seminal vesicles which open into them.

The Ductus Ejaculatorius

The ejaculatory duct (DE) is a median unpaired tube, formed by the union of the two efferent ducts from the mucous glands. It enters the aedeagus sheath and continues posteriorly between the aedeagus arms to the genital aperture at the tip of the aedeagus. Between the aedeagus arms and ventral to the ejaculatory duct is a long tubular gland (AEG), opening into the latter. Anteriorly it stretches beyond the aedeagus sheath.

The Male Genital Armature

The male genitalia consist of the aedeagus with its sheath, situated in an invaginated chamber of the ninth segment.

The aedeagus sheath (Figs. 85, 87, 88, AES) consists of an incomplete chitinous cylinder, a little flattened dorsoventrally, lying in the middle line on the floor of the abdomen. Dorsally the sheath is made into a complete cylinder by a long, subtriangular, thin, chitinous plate (VI), extending from the anterodorsal end of the sheath to the end of the abdomen beneath the anal papilla. At its posteroventral extremity the sheath bears a pair of small processes, ending in three short curved spines. These so-called claspers (CL) probably aid in keeping the aedeagus within the bursa of the female.

The aedeagus (Figs. 85, 86, 87, 88, AED) is a flattened hollow structure, pointed apically and extending backwards on each side in a long chitinous arm (AEDA). It is traversed by the ductus ejaculatorius which opens by a subapical pore (GP). A number of minute papillae can be seen near the tip on each side of the aedeagus (Fig. 87). The aedeagus can be protruded from its sheath for about half its length.

The Digestive System

The alimentary canal in *Monodontomerus*, as in other insects, can be divided into three main regions, the stomodaeum, the mesenteron, and the procto-daeum.

THE STOMODAEUM

The stomodaeum (Figs. 93, 106) includes that part of the alimentary canal which arose by an invagination of the ectoderm from the anterior end of the body, and thus is lined by a chitinous intima. It may be divided into preoral cavity, pharynx, oesophagus, crop, and proventriculus. The wall of the gut in this region consists of an inner chitinous intima (IN), a layer of flat epithelium (ETH), bounded by a basement membrane (BM), and a layer of inner longitudinal and outer circular muscles (LM, CM).

The Preoral Cavity

The preoral cavity (Fig. 106, POR), often erroneously called the buccal cavity, lies below the true mouth opening and is bounded by the mandibles, the labrum, and the labium. Functionally it serves as a mouth. The true mouth lies between the epipharynx and the hypopharynx and leads into the pharynx. The epipharynx bears short hairs called spicules by Snodgrass (11) which project into the mouth.

The Pharynx

The pharynx (Figs. 93, 94, 95, 106, PHY) lies in the anterior part of the head close behind the clypeus and frons, extending dorsally from the mouth to above the level of the antennal sockets, where it turns posteriorly and contracts to the narrow oesophagus. In the floor of the pharynx there is a wide chitinous pharyngeal plate (Figs. 93, 94, PHP) which dorsally is prolonged into two arms, curving upwards in the lateral walls of the pharynx. The intima (Fig. 95) is very thick, and on the anterior wall bears a number of spicules projecting downwards. The epithelium is composed of rather small cuboid cells bounded by a definite basement membrane. The pharynx is well supplied with both dilator and constrictor muscles (Fig. 106, DM, COM) which make it an efficient sucking apparatus.

The Oesophagus

The oesophagus (Figs. 93, 94, 106, OE), a narrow thin-walled tube, passes from the pharynx, between the great cephalic nerve masses and above the body of the tentorium, to the thorax which it traverses to enter the abdomen where it expands into the crop. The intima is thin and the epithelium appears as a few scattered nuclei beneath it. Muscle fibers could not be seen.

The Ingluvies

Owing to the highly developed thoracic musculature, the storage and digestive sections of the gut are located in the abdomen. The ingluvies or crop (Figs. 91, 93, CR) is the chief storage chamber and is formed by the expansion of the oesophagus immediately on passing through the petiole.

The wall of the crop is thin, translucent, and highly distensible. The wall is formed of a layer of thin intima (Fig. 91), which is thrown into folds if the organ is not completely distended, an epithelium revealed only by scattered nuclei, an inner layer of occasional longitudinal muscle fibers, and a well developed outer layer of circular muscles.

The Proventriculus

The crop passes into a short narrow very muscular portion of the gut, the proventriculus (Figs. 92, 93, PVP), which controls the passage of food from the stomodaeum into the mesenteron. The proventriculus projects into the crop as a low mound, often called the calyx, and into the mesenteron a considerably greater distance, forming the stomodaeal or cardiac valve. The proventriculus is triangular in transverse section and the lumen is invaded by three folds or lips, by means of which it can be closed off.

The wall of the proventriculus is thick. A heavy intima (Fig. 92) bearing spicules at the anterior end lines the organ. Only the nuclei of the epithelium can be seen. The muscle layer is well developed and consists of an inner and outer layer of longitudinal muscles enclosing a middle layer of circular muscles.

THE MESENTERON

The mesenteron is the chief digestive and absorptive portion of the gut. It consists of a large stomach or ventriculus (Figs. 93, 96, VT) lying above the ovipositor in segments 6, 7, and 8 of the abdomen. Since it is the endodermal portion of the gut it lacks a chitinous intima. The epithelial cells are large columnar subhexagonal cells, limited by a basement membrane. They have large nuclei which tend to take up a position towards the base of the cells. The cytoplasm is granular and stains deeply. No trace of a peritrophic membrane could be found. There is a weak layer of circular muscles but longitudinal muscles were not seen.

THE PROCTODAEUM

The proctodaeum is the posterior part of the alimentary canal, formed by an ectodermal invagination and consequently lined by a chitinous intima. It consists of the intestine and the rectum.

The Intestine

The mesenteron narrows abruptly to form a poorly marked portion of the intestine, the pylorus, into which the Malpighian tubules open and which, in some insects, is the seat of a well-developed pyloric valve. The intestine (Figs. 93, 98, INT) proceeds forward a short distance and then turns backwards to join the rectum.

The intestine is lined by a thin intima (Fig. 98). The epithelial cells are subcuboid with large nuclei and darkly staining cytoplasm. Circular muscles only could be seen.

The Malpighian tubules (Fig. 93, MAL) vary in number from 16 to 20 in the female and 14 to 16 in the male. In cross section two or three cells can be seen bordering a narrow lumen (Fig. 97).

The Rectum

The intestine expands abruptly to form the rectum (Fig. 93, RC) which in turn leads to the anus in the eversible anal papilla (ALP). The wall of the rectum is very thin and so transparent that the contents can be clearly seen. It consists of a fine intima beneath which appear scattered nuclei of the epithelium. A poorly defined layer without evident striation, but which the author has taken to be the muscular layer, is external to this.

On either side of the opening of the intestine a large convex body is seen in the rectal wall, projecting well into the lumen. These are the rectal glands (RGL). Their structure is difficult to make out. They seem to be composed of a thick inner wall and a thin outer wall, enclosing a narrow lumen. The inner wall is composed of large pyramidal radially-arranged cells with large nuclei and indistinct cell boundaries and is covered with a thin intima where it projects into the rectal lumen. The outer wall is thin and made up of small cells.

THE SALIVARY GLANDS

As many as 10 different kinds of salivary glands have been described in Hymenoptera, though they are not all present in the same species. In *Monodontomerus* only two kinds were found, the thoracic and sublingual.

The thoracic or labial salivary glands (Fig. 93, LGL) are the best developed in Hymenoptera. In *Monodontomerus* they consist of a pair of elongated cylindrical glands lying in the prothorax, each equipped with a duct (LD) which passes forward to the body of the tentorium, where it joins with its fellow to form a common duct passing below the corporotentorium to empty, at the base of the hypopharynx, in a groove, the salivarium (Fig. 106). The duct is stout walled and possesses a chitinous intima which is thickened at regular intervals to form threadlike ridges, projecting into the lumen and resembling the taenidia of tracheae (Fig. 90).

The sublingual glands (Figs. 89, 106, HGL) consist of a number of large cells lying in the hypopharynx and opening by a duct in its floor. In *Harmolita* James (7) has found mandibular glands near the base of each mandible. Pharyngeal glands along the posterior wall of the pharynx have been described in some Hymenoptera. In *Monodontomerus* there are in these positions large cells which are possibly fat cells, especially since no ducts were found in connection with them.

The Respiratory System

Since the comparative anatomy of the respiratory system in the Hymenoptera particularly in the chalcids has been neglected and the terminology is sparse, the various tracheae will be referred to by number rather than by a name which further research might show to be unsuitable.

The tracheal system (Fig. 99) consists essentially of two longitudinal trunks, running from the cervical region to the eighth abdominal segment. The largest tracheae in the body (1) are found in the mesothorax, lying on either side of the middle line and connected anteriorly by the middle connective (CMD). They separate and pass laterally to receive the stigmatic branch (ST1) from the first spiracle (SP1). The trunks then traverse the prothorax where they are joined by the anterior connective (CAT), but they proceed as separate trunks to the head, where each breaks up into a dorsal set of branches and a ventral set of branches. The dorsal branches (2, 3, 4) supply the dorsal region of the head and brain. The ventral branches (5, 6, 7) supply the ventral region of the head and brain, while a trachea to each antenna (8) usually arises from the base of 6.

In the mesothorax there is a pair of lateral trunks (9), lying just inside the pleural wall near the tergopleural junction. They give large branches to the tergosternal muscles and small branches (10, 11) to the first and second wings, respectively. Each joins trachea 1, both anteriorly and posteriorly, and thus forms a ring surrounding the tergosternal muscles. A large dorsal trunk (12) joins 1 and 9 at each end of the ring. The resultant pair of trunks curve upwards and redivide many times to supply the dorsal wing muscles. This pair is joined by a dorsal connective (CDO). A stigmatic branch (ST2) from the propodeal spiracle (SP2) joins the common chamber formed by tracheae 1, 9, and 12. From this chamber a large trunk (13) passes backwards and narrows to enter the petiole and abdomen where it is continued as the main longitudinal trunk of the abdomen (TLA).

The prothoracic leg is supplied by three tracheae. One (14) passes forward from 1 to the prothorax, where it gives branches to the ganglion and then curves posteriorly beneath the furca to enter the coxa. Another (15) arises from the longitudinal trunk in the prothorax near the anterior connective, while a third (16) comes off the anterior common chamber formed by the union of tracheae 1, 9, and 12.

The mesothoracic leg is supplied by a large tracheal branch (17) and a smaller one (18) from 1.

The metathoracic leg is aerated by two branches (19, 20) from 13, and a branch (21) from 9.

In the abdomen tracheae 13 are continued as the main longitudinal trunks (TLA), lying in a dorsolateral position and opening by a stigmatic trunk (ST3) and spiracle (SP3) on the eighth tergum. They give rise to four pairs of highly branched tracheae which aerate the ovaries and fat body in the female and the fat body in the male. From the base of the longitudinal trunks in the female (Fig. 99) and from the stigmatic branch in the male (Fig. 102), a lateral longitudinal branch (22) proceeds forwards supplying the ovaries and fat body, while a posterior branch (23) supplies the terminal portion of the abdomen and rectum.

The paired longitudinal trunks are joined by a posterior connective (CPT) from which a number of tracheae arise. This region is much better developed

in the female and is subject to considerable variation, even on the two sides of the same individual. In the male (Fig. 102) the branches are small and supply the aedeagus. In the female (Fig. 99) the branches are much larger. A branch (24) proceeds forward and aerates the ventral wall of the vagina and probably the poison sacs and adjacent structures. Another branch (25) supplies the common oviduct, accessory glands, and spermatheca and also the abdominal ganglia. Most of the other branches (26) enter the muscles of the ovipositor. A minute trachea (27) passes backwards on the floor of the ovipositor valves and supplies the terminal portions of the latter.

The adult *Monodontomerus* has only three pairs of spiracles though more are present in the larva. The first spiracle is situated in the membrane between the pro- and mesothorax (Figs. 17, 99, SP1). Morphologically it belongs to the mesothorax. The second spiracle (Figs. 23, 38, 99, SP2) opens on the propodeum but belongs to the first abdominal segment. The meta-thoracic spiracle is absent in the adult of *Monodontomerus*. The third spiracle (Figs. 99, 102, SP3) opens on the eighth abdominal segment, to which it belongs. The spiracles usually present on the second to seventh abdominal segments of many insects have disappeared in this form.

The Central Nervous System

The central nervous system (Figs. 100, 102) consists of two great cephalic nerve masses, one above and one below the alimentary canal, forming the cephalic component; three thoracic ganglia, connected by an apparently unpaired nerve cord, forming the thoracic part; and an abdominal component, formed of two ganglia in the female and one in the male.

The Cephalic Nerve Centers

The cephalic nerve centers (Figs. 100, 101, 103, 104, 105, 106, 107) consist of a supraoesophageal ganglion (GDO) or brain, lying above the oesophagus, a suboesophageal ganglion (GVO), lying below it and joined to the former by short thick connectives lateral to the oesophagus. These centers are large and occupy most of the space in the head. The brain is divisible into three regions the protocerebrum, the deutocerebrum, and the tritocerebrum.

The Protocerebrum

The protocerebrum (Figs. 103, 105, 107, 1BR) is composed of a pair of protocerebral lobes interconnected by a median commissural system, the central body (Fig. 103, CB). On each side the protocerebrum is produced into the optic lobes (Figs. 101, 103, 104, OPL), supplying the compound eyes. In section each optic lobe or tract is seen to be formed of three zones, an outer periopticon (POPT), connected to the ommatidia by a layer of postretinal fibers, a middle epiopticon (EOPT), and an inner opticon (OPT).

The paired mushroom bodies or corpora pedunculata (Fig. 103, CP) are buried dorsally in the cortical layer of the protocerebrum. Each consists of two concave discs or caps, forming the calyx (CYX), supported on a bifurcated

stalk or cauliculus, arising ventral to the central body. The concave surface of one cap points posteriorly, that of the other points dorsolaterally; both are filled with darkly stained cortical cells. The pedicel of the mushroom bodies was not seen.

Dorsally, between the corpora pedunculata, are the ocellar lobes which give rise to the ocellar nerves (Figs. 101, 103, 105, 107, NO) supplying the ocelli. The median nerve appears double in section.

The Deutocerebrum

The deutocerebrum (Figs. 104, 105, 107, 2BR) lies in a position antero-ventral to the protocerebrum. In section it has a fasciculated appearance. It consists of a pair of antennary or olfactory lobes which give rise to the antennary nerves (Fig. 101, NA).

The Tritocerebrum

The tritocerebrum (Fig. 105, 3BR) is hidden behind the deutocerebrum so that it is visible only in sections. It consists of a pair of minute lobes giving off a pair of frontal commissures (Figs. 101, 105, NF), connecting it to the frontal ganglion (Fig. 106, GF).

The Suboesophageal Ganglion

The suboesophageal ganglion (Figs. 101, 103, 106, 107, GVO) lies beneath the oesophagus and is joined to the brain by a pair of stout thick commissures. Posteriorly it is connected to the prothoracic ganglion by the ventral nerve cord (Figs. 100, 106, NV). It gives rise to three main nerves supplying the mandibles (Fig. 107, NMD), maxillae (NMX), and labium (NL).

The insect head has been generally regarded as formed of six segments, the three divisions of the brain and triple nature of the suboesophageal ganglion being considered as proof of this. Sollaud and Snodgrass have advanced the theory that the arthropod head is formed from the annelid prostomium plus the first four somites of the body. Thus the protocerebrum and deuto-cerebrum, supplying the eyes and antennae, are taken to be secondary divisions of the prostomial ganglion; the tritocerebrum to be the ganglion of the first true somite, and the suboesophageal ganglion the coalesced nerve centers of the second, third, and fourth somites, bearing respectively the mandibles, maxillae, and labium.

THE THORACIC NERVE CENTERS

The thoracic nerve centers (Fig. 100) are three in number, one to each segment, and are connected by the ventral nerve cord. Both nerve cord and ganglia show evidence of their double nature in section. The meso- and metathoracic ganglia show incipient signs of a coalescence already accom-plished in the bee and the wasp.

The Prothoracic Ganglion

The prothoracic ganglion (GT1) lies on the floor of the prosternum and is supported by the profurca. It gives off a nerve to the prothoracic muscles

and a large one to the leg (NL1), from which a small branch is given off to the muscles of the coxa.

The Mesothoracic Ganglion

The mesothoracic ganglion (GT2) lies in the hind part of the mesothorax, supported on the furca. It is considerably elongated forwards and joins the prothoracic ganglion by a long connective. Between the prothoracic and mesothoracic ganglia, at the level of the prepectus, there is a swelling on the nerve cord which James (7) has termed the mesothoracic accessory ganglion (GAC). It gives rise to two large pairs of nerves. The first pair (NW1) lie close upon the epidermis and pass to the mesothoracic wing giving off branches to the tergosternal muscles on the way. The second pair supply the dorsal longitudinal muscles (ND). The mesothoracic ganglion gives rise to a pair of nerves (NM), supplying the direct wing muscles and probably also sending branches to the indirect wing muscles. The largest nerves are those supplying the legs (NL2). Where these are given off from the ganglion, a nerve supplying the coxal muscles arises.

The Metathoracic Ganglion

The metathoracic ganglion (GT3) lies in the anterior part of the metathorax and propodeum, supported on the metafurca. It consists, at least, of the fused ganglia representing the metathorax and the first two abdominal ganglia, which latter supply the propodeum and the petiole. Its largest nerves are those supplying the hind legs (NL3). Small proximal branches of these supply the coxal muscles. It also bears a small nerve, supplying the hind wing (NW2), and at least two other nerves, supplying the muscles of the propodeum and metathorax. Nerves to the petiole were not found but they would be extremely small.

THE ABDOMINAL NERVE CENTERS

In the female there are two abdominal ganglia connected by a very short commissure and lying dorsal to the common oviduct. The first ganglion (Fig. 100, GA1) lies right upon the common oviduct, the second (GA2) is subspherical and lies posterior to the common oviduct between the oviducts of each side. There is a long nerve cord, connecting the ganglia to those in the thorax. A nerve to the third abdominal segment arises from this connective. The first abdominal ganglion gives off four pairs of visible nerves to segments four, five, six, and seven and thus seems to represent the four fused ganglia of these segments. The second abdominal ganglion supplies the ovipositor and terminal segments of the abdomen and probably represents the last ganglionic mass of the larval nerve chain. Three pairs of nerves arise ventrally and pass forwards to supply the ovipositor and floor of the abdomen. Three pairs pass backwards. One pair (NAT) supplies the terminal floor of the abdomen and the rectum (NR), the others supply the eighth and ninth segments.

In the male there is one central nerve mass (Fig. 102, GA1), situated on the third sternum and probably representing the last six ganglia of the larval

abdomen. It gives off paired nerves to segments three, four, five, six, and seven. It is continued posteriorly as a median nerve, which proceeds backwards to the level of the aedeagus sheath where it divides into two branches, one passing to each side of the sheath and giving off three nerves to the genital armature. Each main branch continues backwards to supply the terminal· portion of the abdomen. Proximally the median nerve is slightly swollen. Just before it divides, the median nerve gives off two pairs of small nerves, the first of which supplies the glandulae mucosae.

Thus there is considerable difference between the abdominal nervous system of the male and female. At first sight it appears that more cephalization has occurred in the male, since there is only one definite nerve center in an anterior position, while the female possesses two such centers in a more posterior position. The author believes that further inspection casts doubt on this view. The posterior position of the female nerve centers is necessary, since the upwardly diverging arms of the ovipositor occupy practically all the space in the anterior part of the abdomen. In the female the nerve to the third abdominal segment arises from the ventral nerve cord, connecting the abdominal with the thoracic nerve centers, and presumably its tracts end in the compound metathoracic ganglion. In the male this nerve arises from the abdominal ganglion. Thus the third thoracic nerve mass in the female consists of the metathoracic ganglion fused with those of the first three abdominal segments and thus exhibits more cephalization than the nerve mass in the male, which consists of the metathoracic ganglion and two abdominal ganglia. In the female the first abdominal ganglion gives rise to four paired nerves supplying segments four to seven, while that of the male supplies the same segments as well as segment three. The second abdominal ganglion, in the female, supplies the eighth segment and those posterior and ennervates the reproductive system and genital armature. It is the author's opinion that this ganglion can be homologized with the median nerve and its swelling in the male, since these also supply the eighth and terminal segments and the reproductive system.

THE VISCERAL NERVOUS SYSTEM

The stomatogastric component of the visceral nervous system consists of a frontal ganglion (Fig. 106, GF) on the anterior surface of the pharynx, connected to the tritocerebral lobes by the frontal commissures (Fig. 105, NF), and a recurrent nerve (NRE) passing backwards on the dorsal surface of the oesophagus to the hypocerebral ganglion just behind the brain.

Acknowledgments

References

1. BUGNION, E. Recherches sur le développement postembryonnaire, l'anatomie et les moeurs de l'*Encyrtus fuscicollis*. Rec. Zool. Suisse : 435-534. 1890.

2. BURKS, B. D. A study of chalcidoid wings. Ann. Entomol. Soc. Am. 31 : 157-160. 1938.

3. HANNA, A. D. The male and female genitalia and the biology of *Euchalcidia caryobori* Hanna (Hymenoptera, Chalcidinae). Trans. Roy. Entomol. Soc. London, 82 : 107-136. 1934.

4. HANNA, A. D. The morphology and anatomy of *Euchalcidia caryobori* Hanna. (Hym. Chalcidinae). Bull. soc. entomol. Egypte, Le Caire, 19 : 326-361. 1935.

5. IMMS, A. D. Observations on the insect parasites of some Coccidae. I. On *Aphelinus mytilaspidis* Le Baron, a chalcid parasite of the Mussel scale (*Lepidosaphes ulmi* L.). Quart. J. Microscop. Sci. London, 61 : 217-274. 1916.

6. IMMS, A. D. Observations on the insect parasites of some Coccidae. II. On the Chalcid parasites of *Lecanium capreae*. Quart. J. Microscop. Sci. London, 63 : 293-374. 1918.

7. JAMES, H. C. The anatomy of a British phytophagous Chalcidoid of the genus *Harmolita* (*Isosoma*). Proc. Zool. Soc. London : 75-182. 1926.

8. LEONARD, M. D. A list of the insects of New York. Cornell Univ. Agr. Expt. Sta. Mem. 101. 1926.

9. MUESEBECK, C. F. W. *Monodontomerus aereus* Walker, both a primary and a secondary parasite of the brown-tail moth and the gypsy moth. J. Agr. Research, 43 : 445-460. 1931.

10. SNODGRASS, R. E. The thorax of Hymenoptera. Proc. U.S. Nat. Museum, 39 : 37-91. 1911.

11. SNODGRASS, R. E. Principles of insect morphology. McGraw-Hill Book Co., Inc., New York. 1935.

NOTE: *Figs. 1-107 will be found on pp. 265-281.*

EXPLANATION OF FIGURES

FIG. 1. *Dorsal view of the head.* × 55.

FIG. 2. *Lateral view of the head and mouth parts.* × 55.

FIG. 3. *Frontal view of the head.* × 55.

FIG. 4. *Frontal view of the head, specimen cleared and frons removed to show the tentorium.* × 55.

FIG. 5. *Posterior view of the head and mouth parts.* × 55.

FIG. 6. *Posterior view of the head, specimen cleared to show the tentorium.* × 55.

A, articulation; AN, antennifer; AS, antennal suture; ASO, antennal socket; AT, anterior tentorial pit; ATA, anterior tentorial arm; B.S, basal segment; CD, cardo; CLP, clypeus; CT, corporotentorium; E, compound eye; ES, epistomal suture; FEX, frontal excavation; FOR, foramen magnum; FR, frons; GA, galea; GE, gena; GU, gula; ITR, internal ridge of the tentorium; LB, labrum; LC, lacinia; LIG, ligula; LO, lateral ocellus; LSC, ligular sclerite; MD, mandible; MO, median ocellus; MT, mentum; OC, occiput; OCC, occipital condyle; OCS, occipital suture; PDC, pedicel; PGE, postgena; POC, post-occiput; POCS, postoccipital suture; PRTL, parietal region; PT, posterior tentorial pit; PTA, posterior tentorial arm; RS, ring segment; SCP, scape; SGE, subgena; SGS, sub-genal suture; SMT, submentum; SOS, subocular suture; ST, stipes; TL, tentorial lamella; VX, vertex.

FIG. 7. *Posterior view of the right maxilla.* × 100.

FIG. 8. *Medial view of the left maxilla.* × 100.

FIG. 9. *Lateral view of the right maxilla.* × 100.

FIG. 10. *Posterior view of the labium.* × 100.

FIG. 11. *Anterior view of the labium.* × 100.

FIG. 12. *Lateral view of the labium.* × 100.

FIG. 13. *Anterior view of the right mandible.* × 100.

FIG. 14.   *Posterior view of the right mandible.*   × *100.*

FIG. 15.   *Sixth segment of the flagellum of the female antenna.*   × *100.*

A, articulation; C, condyle; CD, cardo; GA, galea; HPH, hypopharynx; HSC, sclerite of the hypopharynx; LC, lacinia; LIG, ligula; LSC, ligular sclerite; MT, mentum; SP, sensory pit; ST, stipes.

FIG. 16.   *Lateral view of the prothorax.*   × *55.*

FIG. 17.   *Posterior view of the prothorax, showing the first spiracle.*   × *45.*

FIG. 18.   *Ventral view of the prothorax.*   × *45.*

FIG. 19.   *Dorsal view of the endoskeleton of the thorax.   The specimen has been cleared and the nota removed.*   × *55.*

FIG. 20.   *Posterodorsal view of the prothorax of a cleared specimen.*   × *55.*

FIG. 21.   *Proepisternum and coxa, the former flattened in one plane.*   × *55.*

FIG. 22.   *Ventral view of the cleared prothorax with the head attached.*   × *55.*

A, articulation; AFU, arm of the furca; CV, cervical sclerite; CX, coxa; EPS, episternum; FA, arch of the furca; IPR, pleural ridge; IRP, internal ridge of the prepectus; MFU, manubrium of the furca; MTR, reduplication of the metasternum; OCC, occipital condyle; PHA, articulation of the pronotum with the head; PMA, articulation of the pronotum with the mesothorax; PRP, prepectus; PRT, protergum; PSR, reduplication of the prosternum; PWP, pleural wing process; SP1, first spiracle; STN', STN'', STN''', pro-, meso-, metasternum; ST1, first stigmatic trunk.

FIG. 23.   *Lateral view of the thorax.*   × *50.*

FIG. 24.   *Ventral view of the thorax showing the position of the coxae.*   × *50.*

FIG. 25.   *Dorsal view of the thorax.*   × *50.*

FIG. 26.   *Ventral view of the thorax.*   × *50.*

AAP, anterior alar plate; AB, postalar bridge; ANP, anterior notal wing process; CX, coxa; CXC, coxal cavity; D, dimple of the mesepimeron; EPM, epimeron; EPS, episternum; FP, furcal pit; MN, metanotum; MPA, articulation of the mesothorax with the prothorax; MSR, reduplication of the mesosternum; MTR, reduplication of the metasternum; PAP, posterior alar plate; PAR, parapsidal plate; PD, propodeum; PER, external rim of the parapsis; PF, parapsidal furrow; PNP, posterior notal wing process; PRP, prepectus; PRT, protergum; PS, pleural suture; PSCT, postscutum; PSS, suture of the postscutum; PWP, pleural wing process; SCL, scutellum; SCS, subcostal scale; SCT, scutum; SFL, postscutal flange; SP2, second spiracle; STN, sternum; TEG, tegula; TP, triangle of the prepectus; TR, trochanter; TSCS, transscutellar suture; TSS, transscutal suture; VS, scutoscutellar suture; 2-3S, abdominal sterna; 2-5T, abdominal terga; 2TR, second trochanter.

FIG. 27.   *Lateral view of the detached and cleared mesothorax.*   × *55.*

FIG. 28.   *Dorsal view of the detached and cleared mesothorax.*   × *55.*

FIG. 29.   *Ventral view of the cleared mesonotum.*   × *55.*

A, articulation; AAP, anterior alar plate; AB, postalar bridge; ACS, antecostal suture; ANP, anterior notal wing process; D, dimple of the mesepimeron; EP, internal spine of the epimeron; EPM, epimeron; EPS, episternum; IPR, pleural ridge; IWR, internal ridge supporting the wing process; MPA, articulation of the mesothorax with the prothorax; MSR, reduplication of the mesosternum; MSRA, process of the reduplication of the mesosternum; PAP, posterior alar plate; PAR, parapsidal plate; PER, external rim of parapsis; PF, parapsidal furrow; PFL, parapsidal flange; PN, mesopostnotum; PNP, posterior notal wing process; PR, internal parapsidal ridge; PRP, prepectus; PS, pleural suture; PSCT, postscutum; PSR, parapsidal submarginal ridge; PSS, suture of the postscutum; PWP, pleural wing process; SCL, scutellum; SCT, scutum; SFL, postscutal flange; SPS, pseudophragma of the postscutum; TEG, tegula; TSCS, transscutellar suture; TSS, transscutal suture; VR, internal ridge of the scutoscutellar suture; VS, scutoscutellar suture; X, articulating sclerite of the metanotum; 1PH, prephragma; 2PH, postphragma.

FIG. 30.   *Posterodorsal view of the cleared mesopectus showing the endoskeleton.*   × *55.*

FIG. 31.   *Ventral view of the mesothorax.*   × *55.*

FIG. 32.   *Lateral view of the cleared mesopectus showing the endoskeleton.*   × *55.*

Fig. 33. *Anteroventral view of the cleared mesonotum, showing the postnotum and its phragmata.* × 55.

Fig. 34. *Ventroposterior view of the metathorax and propodeum.* × 55.

A, *articulation;* AB, *postalar bridge;* AFU, *furcal arm;* D, *dimple of the mesepimeron;* CX, *coxa;* CXC, *coxal cavity;* EP, *internal spine of the epimeron;* EPM, *epimeron;* EPS, *episternum;* FA, *furcal arch;* FP, *furcal pit;* IPR, *pleural ridge;* IRP, *internal ridges of the prepectus;* IWR, *internal ridge of the wing process;* MFU, *furcal manubrium;* MPA, *articulation of the mesothorax with the prothorax;* MSR, *reduplication of the mesosternum;* MSRA, *process of the reduplication of the mesosternum;* MTR, *reduplication of the metasternum;* PAP, *posterior alar plate;* PAR, *parapsidal plate;* PD, *propodeum;* PN, *mesopostnotum;* PNP, *posterior notal wing process;* PPS, *pseudophragma of the postnotum;* PRP, *prepectus;* PS, *pleural suture;* PSCT, *postscutum;* PWP, *pleural wing process;* SCL, *scutellum;* SPS, *pseudophragma of the postscutum;* STN, *sternum;* TEG, *tegula;* TP, *triangle of prepectus;* TSS, *transscutal suture;* VR, *internal ridge of the scutoscutellar suture;* 1PH, *prephragma;* 2PH, *postphragma;* 2S, 3S, *abdominal sterna;* 3T, *abdominal tergum.*

Fig. 35. *Dorsal view of the petiole.* × 55.

Fig. 36. *Lateral view of the petiole.* × 55.

Fig. 37. *Anterior view of the petiole.* × 55.

Fig. 38. *Dorsal view of the metathorax and propodeum.* × 55.

Fig. 39. *Anteroventral view of the cleared metathorax and propodeum.* × 55.

Fig. 40. *Posterior view of the cleared metathorax and propodeum.* × 55.

Fig. 41. *Anterior view of the cleared metathorax and propodeum.* × 55.

A, *articulation;* AFU, *furcal arm;* ANP, *anterior notal wing process;* CX, *coxa;* CXC, *coxal cavity;* EPM, *epimeron;* EPS, *episternum;* FOP, *foramen of the propodeum;* INP, *inflection of the metapectus;* IPR, *pleural ridge;* MFU, *furcal manubrium;* MN, *metanotum;* MTR, *reduplication of the metasternum;* PD, *propodeum;* PNP, *posterior notal wing process;* PS, *pleural suture;* PWP, *pleural wing process;* SP2, *second spiracle;* STN, *sternum;* 3S, *abdominal sternum.*

Fig. 42. *Dorsal view of the pretarsus.* × 250.

Fig. 43. *Ventral view of the pretarsus.* × 250.

Fig. 44. *Lateral view of the pretarsus.* × 250.

Fig. 45. *Antenna of the male.* × 55.

Fig. 46. *Antenna of the female.* × 55.

Fig. 47. *Reticulation and setae of the scutum.* × 250.

Fig. 48. *Anterior view of the right prothoracic leg.* × 35.

Fig. 49. *Anterior view of the right mesothoracic leg.* × 35.

Fig. 50. *Anterior view of the right metathoracic leg.* × 35.

AR, *arolium;* BS, *basal segment;* CA, *camera;* CC, *calcar;* CX, *coxa;* FM, *femur;* OR, *orbicula;* PDC, *pedicel;* PTAR, *pretarsus;* RS, *ring segment;* SCP, *scape;* TAR, *tarsus;* TB, *tibia;* TR, *trochanter;* UN, *unguis;* UNG, *unguitractor;* 2TR, *second trochanter.*

Fig. 51. *Lateral view of the coxopleural articulation of the prothoracic leg.* × 100.

Fig. 52. *Lateral view of the coxopleural articulation of the mesothoracic leg.* × 100.

Fig. 53. *Lateral view of the coxopleural articulation of the metathoracic leg.* × 100.

Fig. 54. *Dorsolateral view of the coxotrochanteral and trochanterofemoral articulations of the prothoracic leg.* × 100.

Fig. 55. *Ventral view of the coxotrochanteral and trochanterofemoral articulations of the mesothoracic leg.* × 100.

Fig. 56. *Ventral view of the coxotrochanteral and trochanterofemoral articulations of the metathoracic leg.* × 100.

Fig. 57. *Dorsal view of the tibiofemoral articulation of the prothoracic leg.* × 100.

Fig. 58. *Dorsal view of the tibiofemoral articulation of the mesothoracic leg.* × 100.

Fig. 59. *Dorsal view of the tibiofemoral articulation of the metathoracic leg.* × 100.

Fig. 60. *Lateral view of the tibiotarsal articulation of the prothoracic leg.* × 100.

FIG. 61. *Lateral view of the tibiotarsal articulation of the mesothoracic leg.* × *100.*

FIG. 62. *Tibiotarsal joint showing the condyle.* × *100.*

FIG. 63. *Lateral view of the tibiotarsal articulation of the metathoracic leg.* × *100.*

A, articulation; C, condyle; CC, calcar; CX, coxa; EPS, episternum; FM, femur; PD, propodeum; TAR, tarsus; TB, tibia; TR, trochanter; 2TR, second trochanter.

FIG. 64. *Frenulum.* × *250.*

FIG. 65. *Mesothoracic wing.* × *40.*

FIG. 66. *Metathoracic wing.* × *40.*

FIG. 67. *Dorsal view of the axillary sclerites of the mesothoracic wing.* × *90.*

FIG. 68. *Dorsal view of the axillary sclerites of the metathoracic wing.* × *90.*

FIG. 69. *Lateral view of the thorax with the wings elevated to show the articulations with the pleura.* × *80.*

ANP, anterior notal wing process; AX1, AX2, AX3, axillaries; BAL, basalare; CU', first cubital branch; EPM, epimeron; EPS, episternum; FL, frenulum; M, median vein; M', basal sclerite of the median vein; MV, marginal vein; PD, propodeum; PNP, posterior notal wing process; PMV, postmarginal vein; PSCT, postscutum; PWP, pleural wing process; R, radial vein; R', first radial branch; SC, subcosta; SCL, scutellum; SCS, subcostal scale; SMV, submarginal vein; SP2, second spiracle; STV, stigmal vein; TEG, tegula; WR, sclerotized rib of the mesothoracic wing; X, articulating sclerite of the metanotum.

FIG. 70. *Lateral view of the female abdomen with the sternites depressed and the sting revealed.* × *30.*

FIG. 71. *Lateral view of the male abdomen with the aedeagus exserted.* × *30.*

FIG. 72. *Dorsal view of the female abdomen.* × *30.*

FIG. 73. *Ventral view of the female abdomen.* × *30.*

FIG. 74. *Dorsal view of the male abdomen with the aedeagus exserted.* × *30.*

FIG. 75. *Ventral view of the male abdomen with the aedeagus exserted.* × *30.*

AED, aedeagus; AES, aedeagus sheath; ALP, anal papilla; CX, coxa; CXC, coxal cavity; EPS, episternum; MTR, reduplication of the metasternum; PAL, sensory palp; PD, propodeum; SPL, sensory plate; STY, stylet; STYS, stylet sheath; 2-9S, abdominal sterna; 2-10T, abdominal terga.

FIG. 76. *Dorsal view of the cleared ovipositor.* × *55.*

FIG. 77. *Ventral view of the proximal end of the cleared ovipositor with the plates spread.* × *55.*

FIG. 78. *Inside view of the right half of the cleared ovipositor with the parts spread.* × *55.*

FIG. 79. *Lateral view of the terminal portion of the stylet sheaths showing the teeth.* × *250.*

FIG. 80. *Transverse section of the stylets and stylet sheaths.* × *500.*

FIG. 81. *The sclerite of the vagina.* × *55.*

FIG. 82. *Lateral view of the cleared ovipositor.* × *55.*

A, articulation; ALP, anal papilla; ET, egg-tube; FP, fulcral plate; IP, inner plate; IPB, bridge of the inner plate; IPP, pivoting sclerite of the inner plate; IPR, rib of the inner plate; OP, outer plate; OPR, rib of the outer plate; PAL, sensory palp; RP, rotatory process; RPR, transverse ribs of the rotatory processes; SPL, sensory plate; STY, stylet; STYS, stylet sheath; 9-10T, abdominal terga.

FIG. 83. *The female reproductive system from a dorsal aspect, the ovaries being displaced outwards and backwards.* × *55.*

FIG. 84. *Dorsal view of the poison apparatus.* × *55.*

FIG. 85. *Dorsal view of the male reproductive system.* × *55.*

FIG. 86. *Dorsal view of the aedeagus, cleared and removed from the sheath.* × *55.*

FIG. 87. *Dorsal view of the aedeagus and its sheath.* × *55.*

FIG. 88. *Ventral view of the aedeagus and its sheath.* × *55.*

ACG, acid gland; ACR, reservoir of acid gland; AED, aedeagus; AEDA, aedeagus arm; AES, aedeagus sheath; AGS, accessory glands of spermatheca; ALG, alkaline gland; CH,

chorion; CL, clasper; COV, common oviduct; DE, ductus ejaculatorius; EG, egg; FE, follicular epithelium; FP, fulcral plate; GER, germarium; GM, glandula mucosa; GP, genital pore; IP, inner plate; NC, nurse cells; OP, outer plate; OV, oviduct; OVL, ovariole; SPL, sensory plate; SPM, spermatheca; STY, stylet; STYS, stylet sheath; TT, testis; VAG, vagina; VD, vas deferens; VI, valva interna; VIT, vitellarium; VSC, sclerite of the vagina; VSM, vesicula seminalis; 1AG, primary accessory gland; 2AG, secondary accessory gland; 9T, 10T, abdominal terga.

FIG. 89.　*Section of the salivary gland of the hypopharynx.*　✕ *500.*

FIG. 90.　*Sagittal section of the duct of the thoracic salivary gland.*　✕ *500.*

FIG. 91.　*Transverse section of the crop.*　✕ *500.*

FIG. 92.　*Transverse section of the proventriculus.*　✕ *500.*

FIG. 93.　*Dorsal view of the alimentary canal.*　✕ *55.*

FIG. 94.　*Lateral view of the pharynx showing the pharyngeal plate.*　✕ *55.*

FIG. 95.　*Sagittal section of the inner wall of the pharynx.*　✕ *500.*

FIG. 96.　*Coronal section of the ventriculus.*　✕ *500.*

FIG. 97.　*Transverse section of a Malpighian tubule.*　✕ *500.*

FIG. 98.　*Transverse section of the intestine.*　✕ *500.*

ALP, anal papilla; BM, basement membrane; CM, circular muscles; CR, crop; CX, coxa; EPH, epipharynx; ETH, epithelium; FAT, fat; IN, intima; INT, intestine; LB, labrum; LD, duct of the labial salivary gland; LGL, labial salivary gland; LM, longitudinal muscles; MAL, Malpighian tubules; OE, oesophagus; OP, outer plate; PHP, pharyngeal plate; PHY, pharynx; PVT, proventriculus; RC, rectum; RGL, rectal gland; SPL, sensory plate; VT, ventriculus; 3-8S, abdominal sterna; 9-10T, abdominal terga.

FIG. 99.　*Dorsal view of the tracheal system of the female.*　✕ *55.*

CAT, anterior connective; CDO, dorsal connective; CMD, middle connective; CPT, posterior connective; CX, coxa; SP1, 2, 3, spiracles; STY, stylets; ST1, 2, 3, stigmatic branches; TLA, longitudinal abdominal trunk; 3-8S, abdominal sterna; 2T, 8T, abdominal terga.

FIG. 100.　*Dorsal view of the nervous system of the female.*　✕ *55.*

FIG. 101.　*Frontal aspect of the brain.*　✕ *55.*

FIG. 102.　*Dorsal view of the tracheal and nervous systems in the male abdomen.*　✕ *55.*

AES, aedeagus sheath; CPT, posterior connective; CX, coxa; GAC, accessory ganglion; GA1, GA2, abdominal ganglia; GDO, supraoesophageal ganglion; GT1, 2, 3, thoracic ganglia; GVO, suboesophageal ganglion; HGL, sublingual salivary gland; LD, duct of the labial salivary glands; NA, antennary nerve; NAT, nerve to the terminal floor of the abdomen; ND, nerve to the dorsolongitudinal muscles; NF, frontal commissure; NL1, 2, 3, leg nerves; NM, nerve to the direct wing muscles; NMD, mandibular nerve; NOC, ocellar nerve; NR, nerve to the rectum; NW1, 2, nerves to the wings; OP, outer plate; OPL, optic lobe; SP3, spiracle; STY, stylet; ST3, stigmatic trunk; TLA, longitudinal abdominal trunk; 3-8S, abdominal sterna; 2T, 8T, abdominal terga.

FIG. 103.　*Transverse section of the brain.*　✕ *90.*

FIG. 104.　*Coronal section of the brain.*　✕ *90.*

FIG. 105.　*Sagittal section of the brain.*　✕ *90.*

FIG. 106.　*Sagittal section of the brain showing the pharynx and oesophagus.*　✕ *90.*

FIG. 107.　*Sagittal section of the brain.*　✕ *90.*

CB, central body; COM, constrictor muscles; CP, mushroom body; CYX, calyx; DM, dilator muscles; EOPT, epiopticon; EPH, epipharynx; GDO, supraoesophageal ganglion; GF, frontal ganglion; GVO, suboesophageal ganglion; HGL, sublingual gland; HPH, hypopharynx; LB, labrum, LD, duct of labial salivary glands; LIG, ligula; MO, median ocellus; NF, frontal commissure; NL, labial nerve; NMD, mandibular nerve; NMX, maxillary nerve; NOC, ocellar nerve; NRE, recurrent nerve; NV, ventral nerve cord; OCC, occipital condyle; OE, oesophagus; OPT, opticon; PHY, pharynx; PQPT, periopticon; POR, preoral cavity; 1BR, protocerebrum; 2BR, deutocerebrum; 3BR, tritocerebrum.
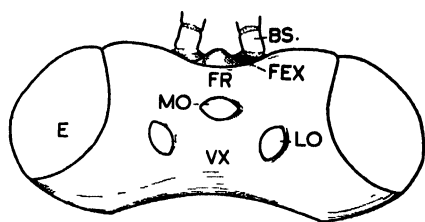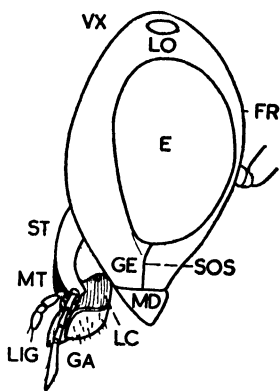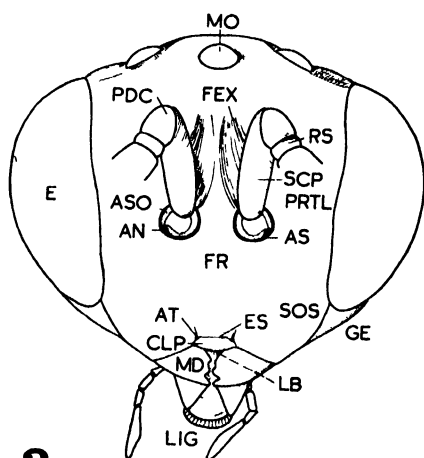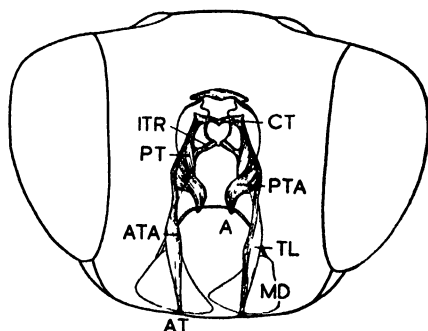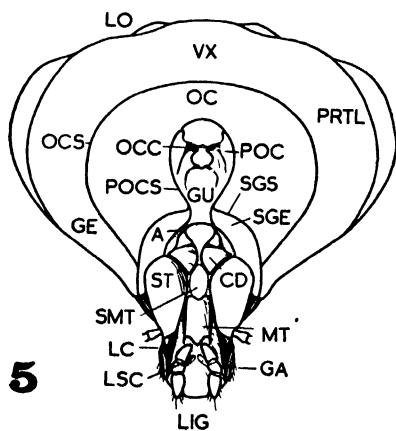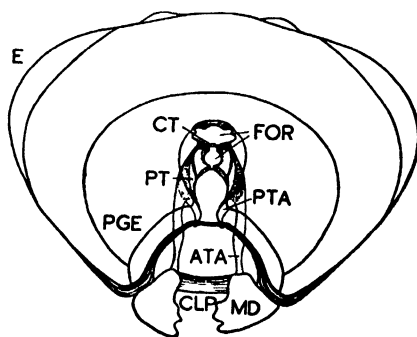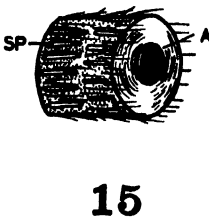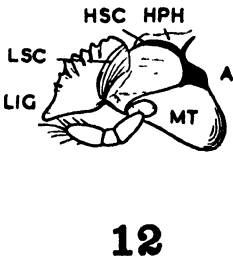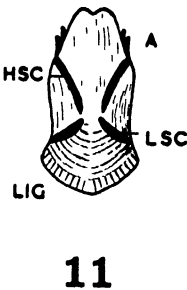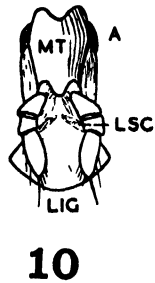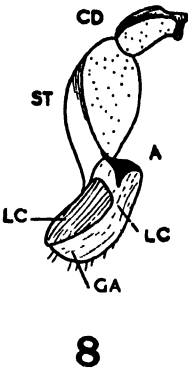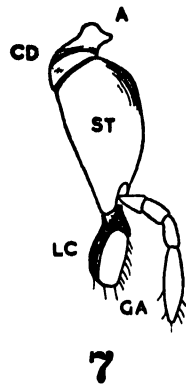
FIGS. 1 TO 6.

FIGS. 7 TO 15.
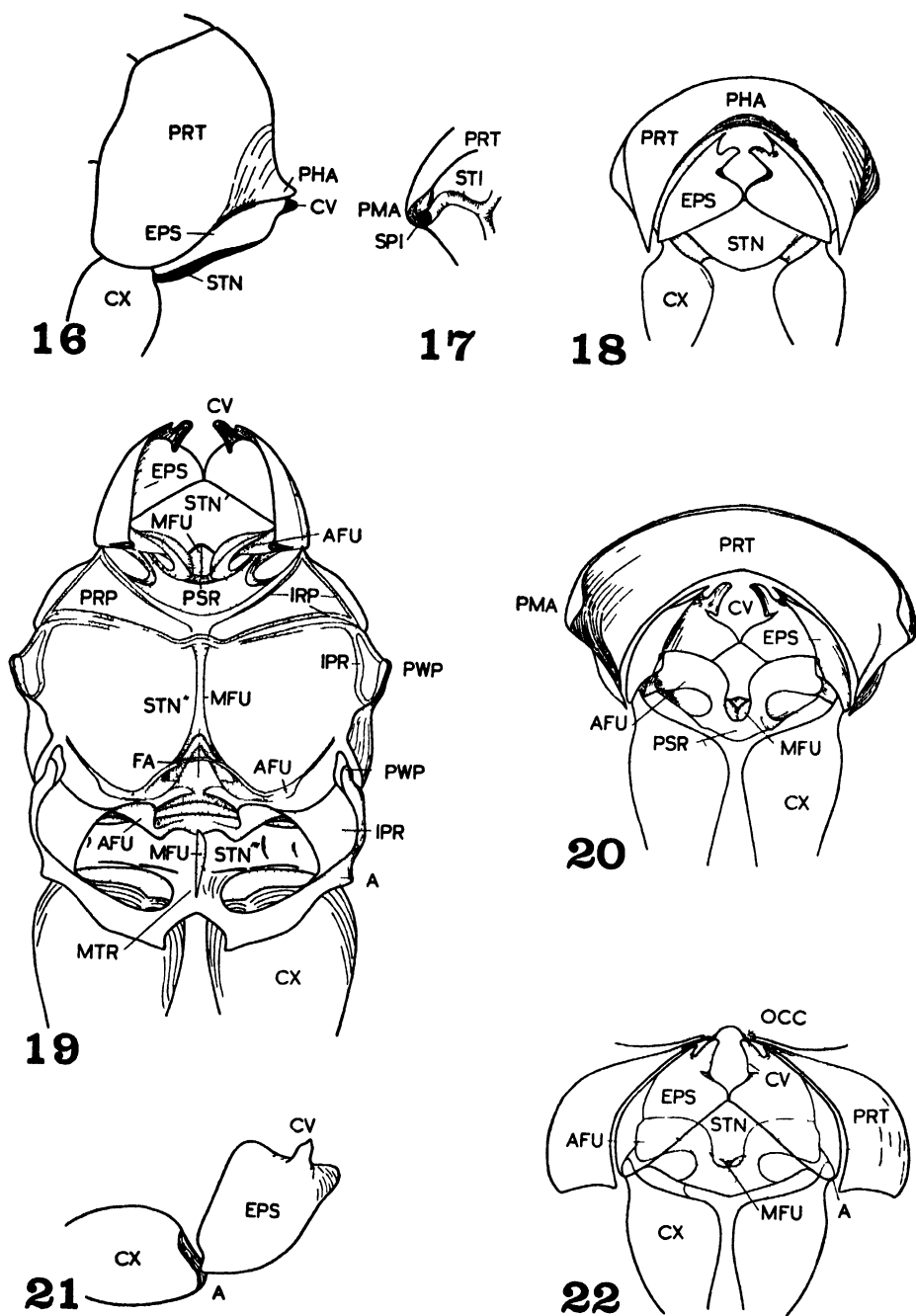
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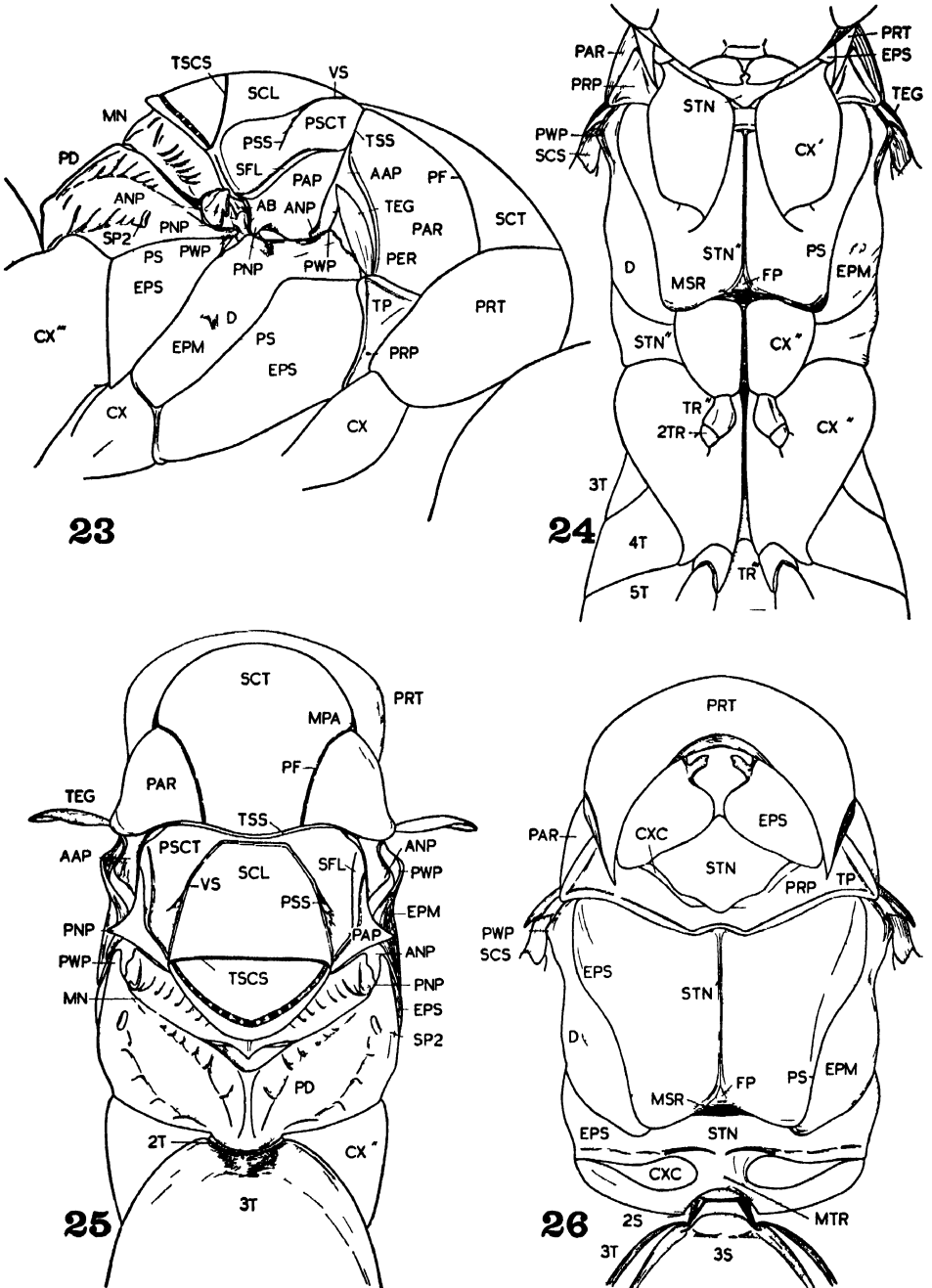
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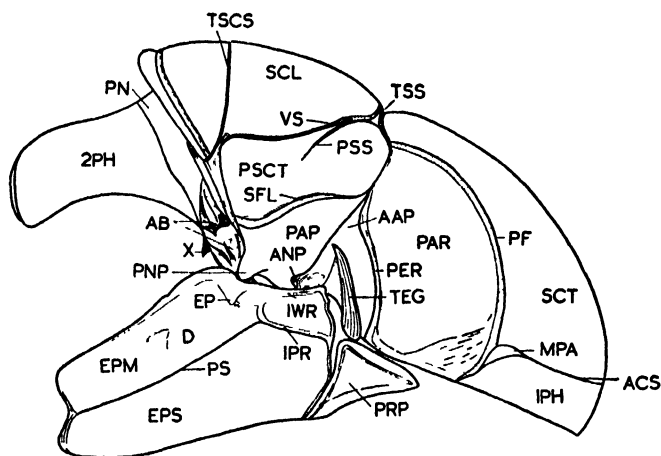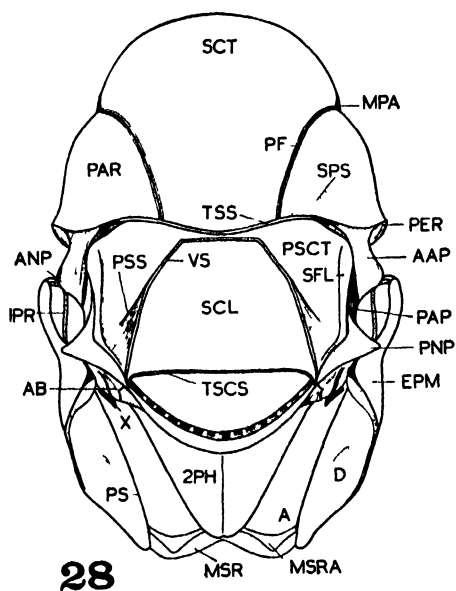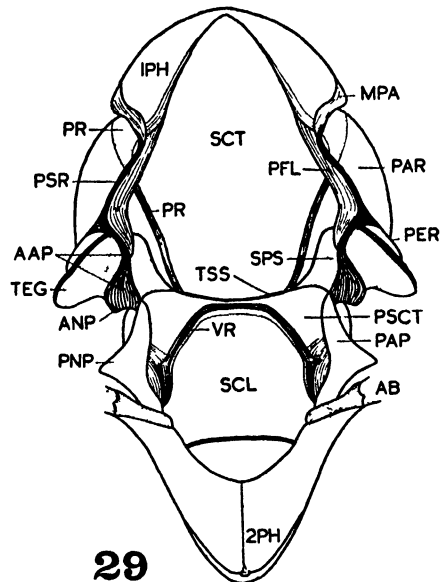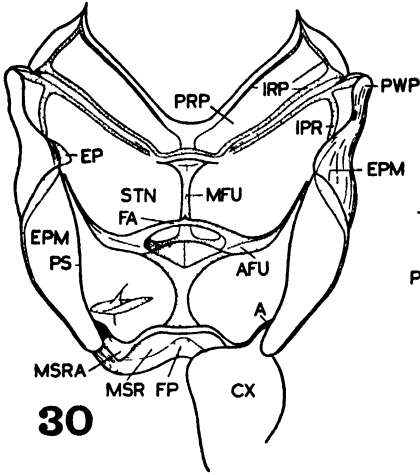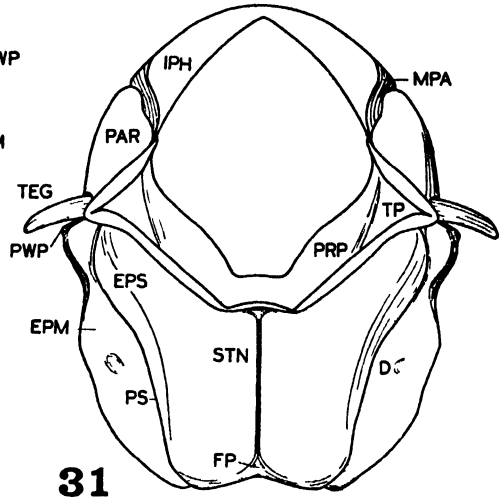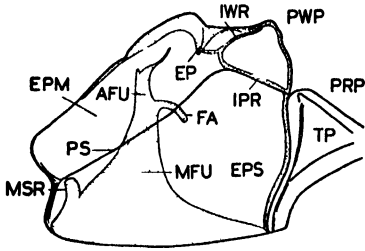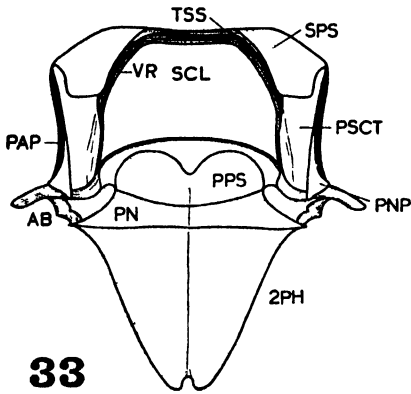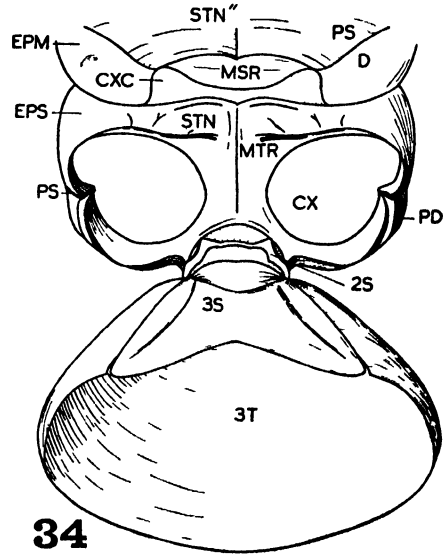
FIGS. 27 TO 29.

FIGS 30 TO 34
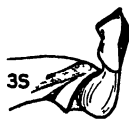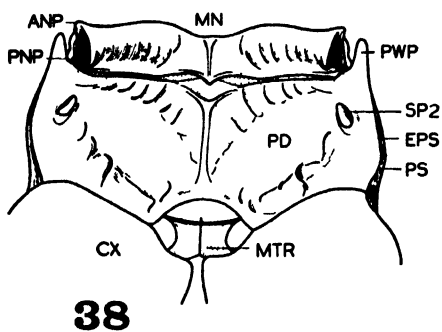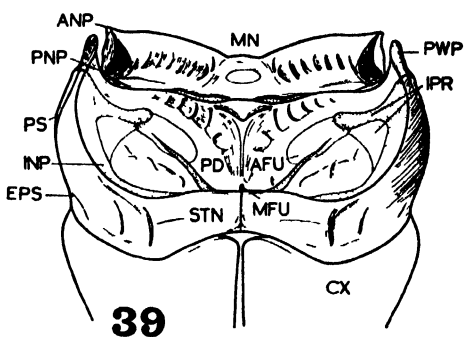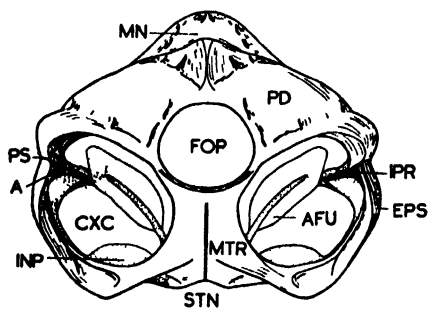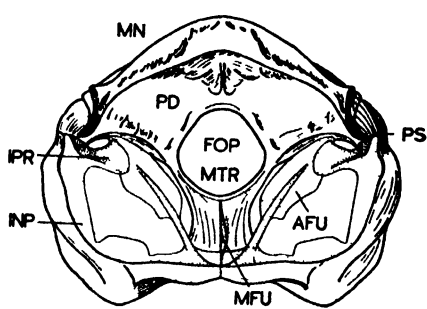
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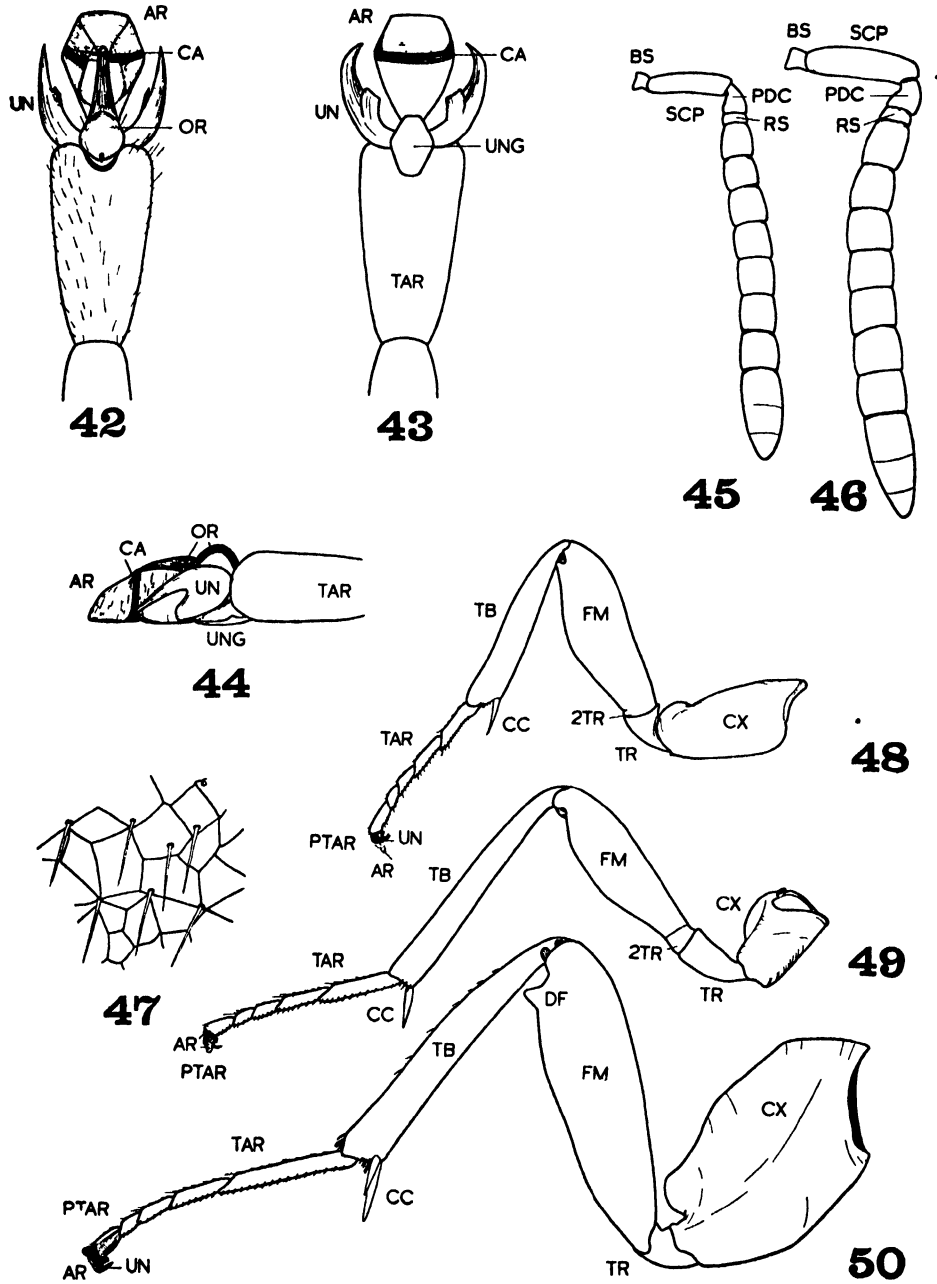
FIGS. 42 TO 50.
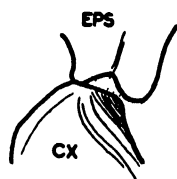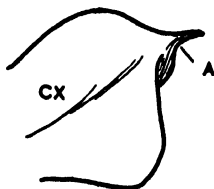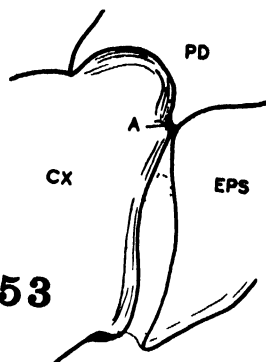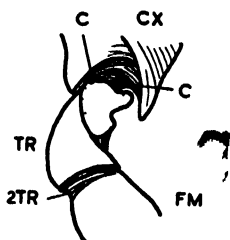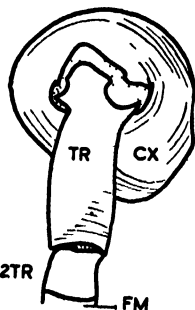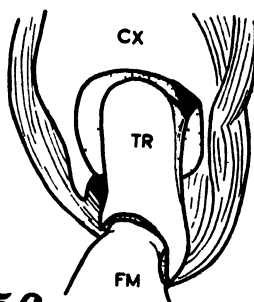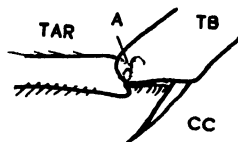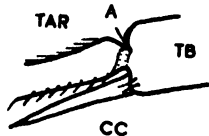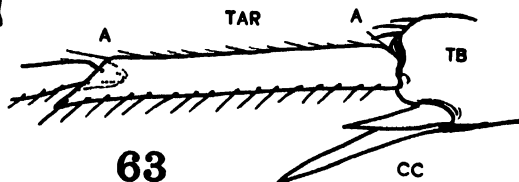
FIGS. 51 TO 63.

FIGS. 64 TO 69.

FIGS. 70 TO 75.
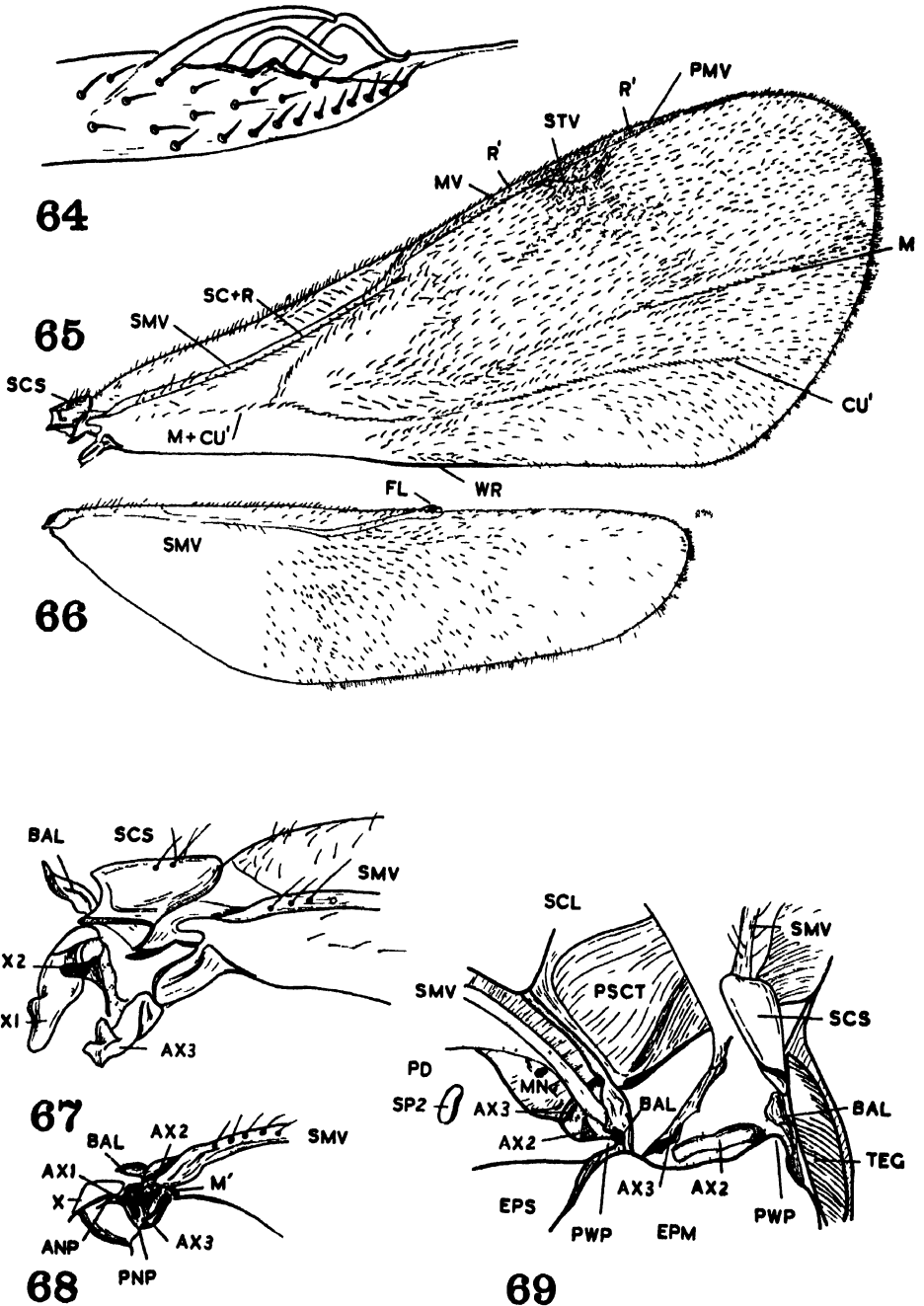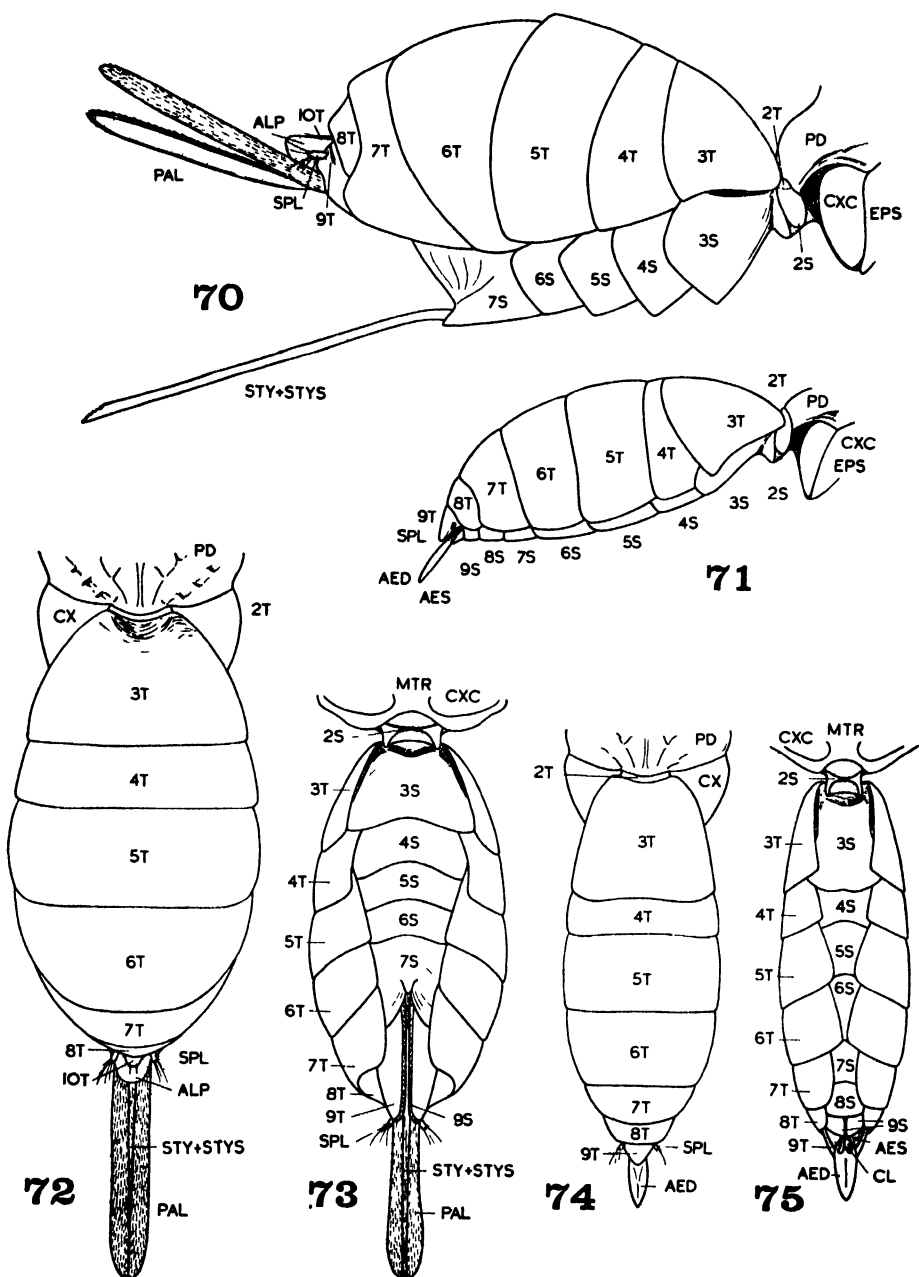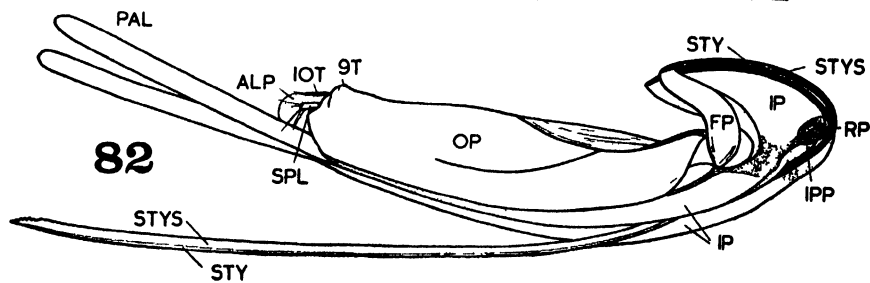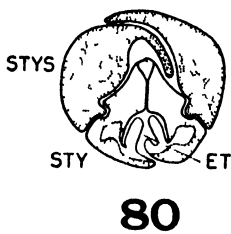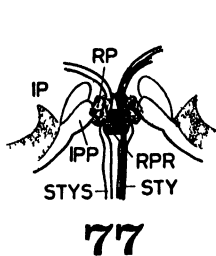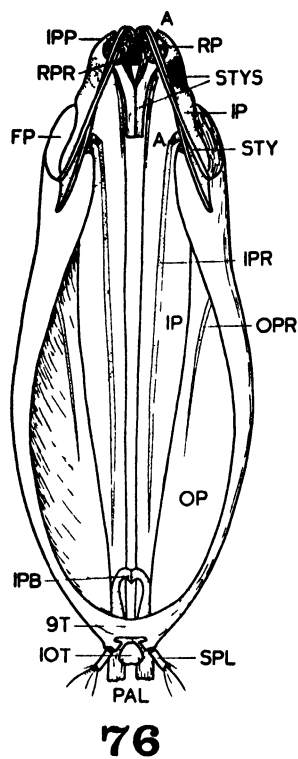
FIGS. 76 TO 82.
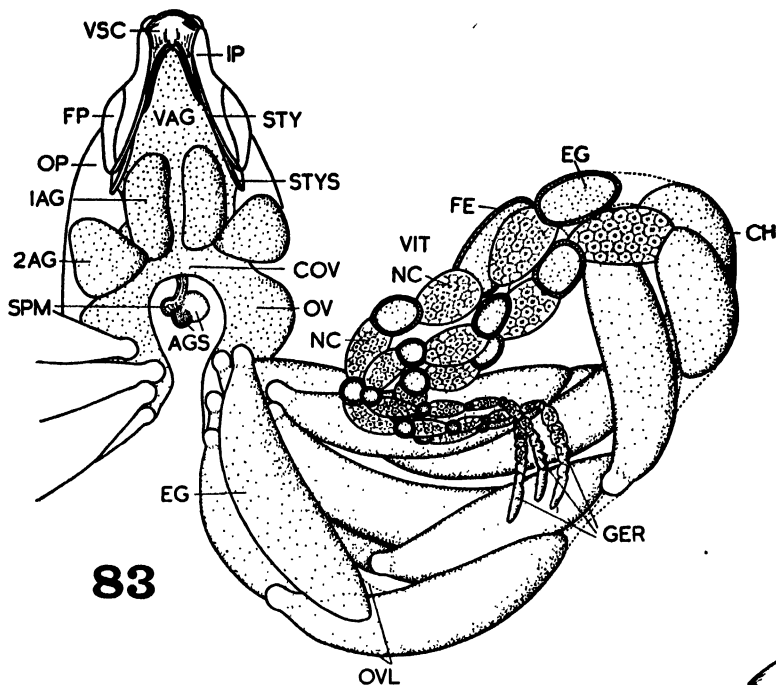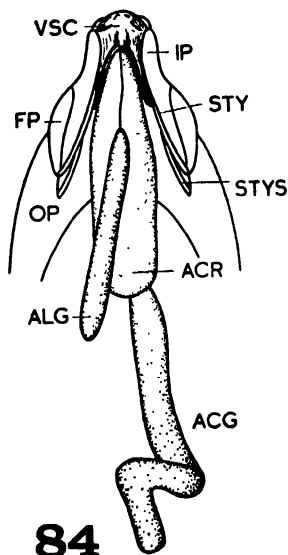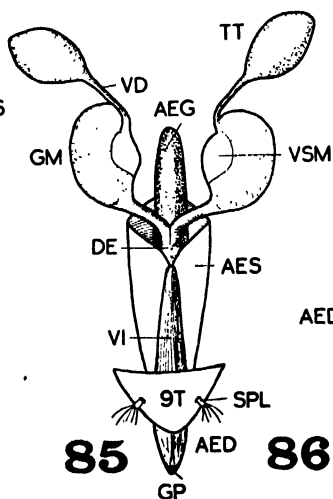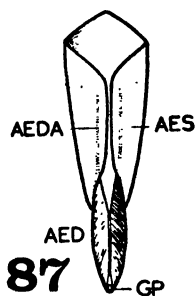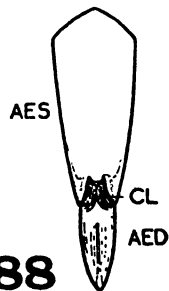
FIGS. 83 TO 88.

FIGS. 89 TO 98.

99

FIG. 99.

GDO

OPL

GTI

NLI

GAC   ND   NWI

NM

GT2

NL2

NW2

GT3

NL3

2T

3S

STY

CX

4S

5S

6S

OP   GAI

7S

GA2   OP

8S

NAT   NR

8T

100

NOC

GDO

NA

GVO   NF

NMD   LD

HGL

OPL

101

3S   GAI

4S   TLA

5S

22

6S

AES

7S   22

ST3   CPT

23   SP3

8T

102
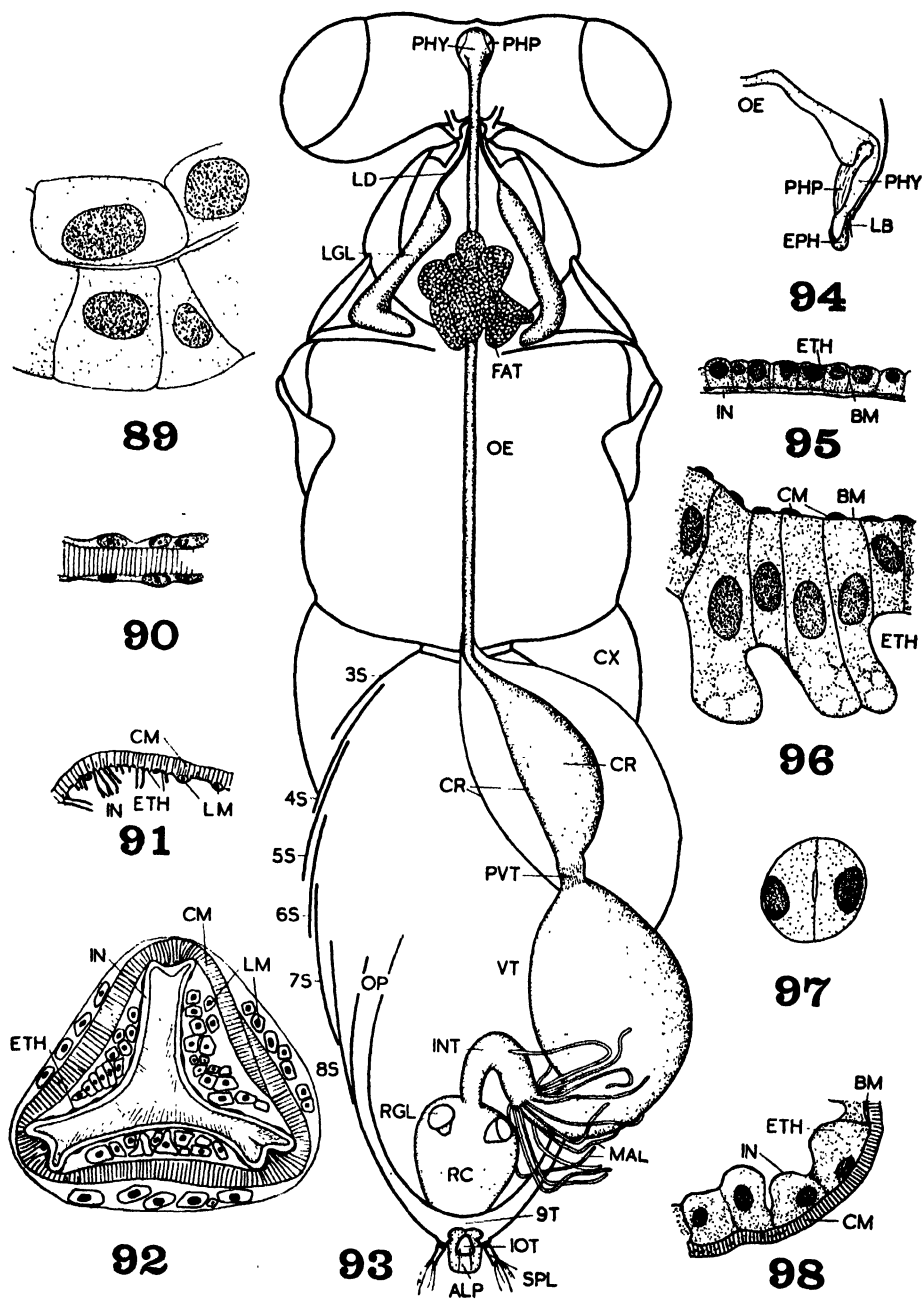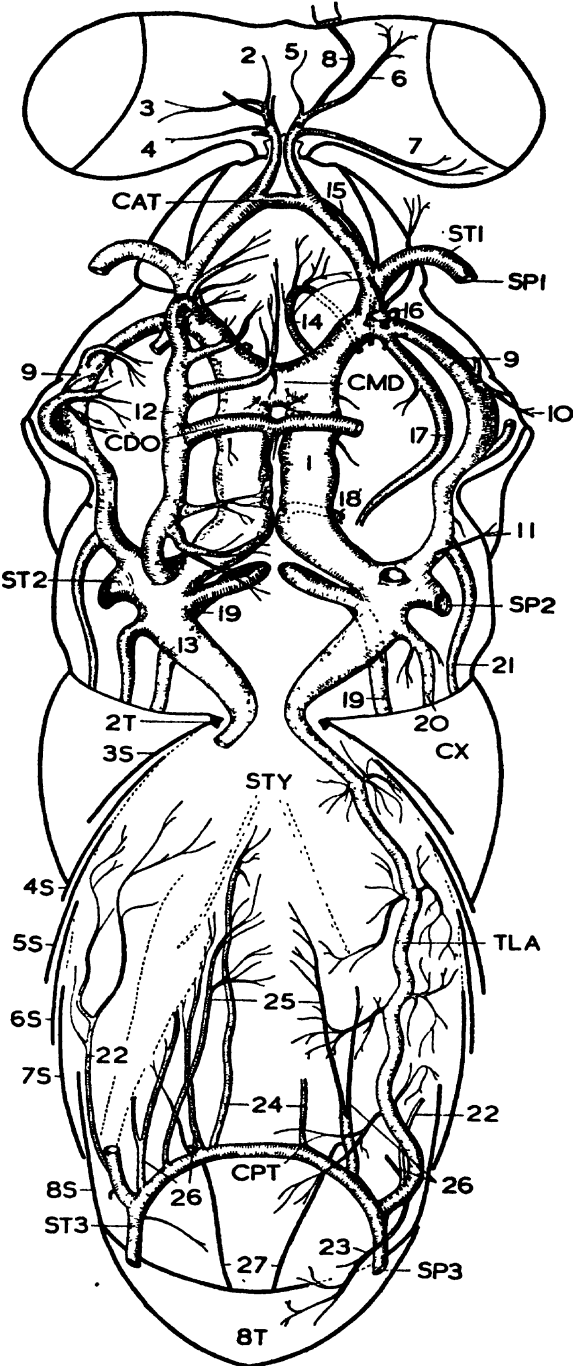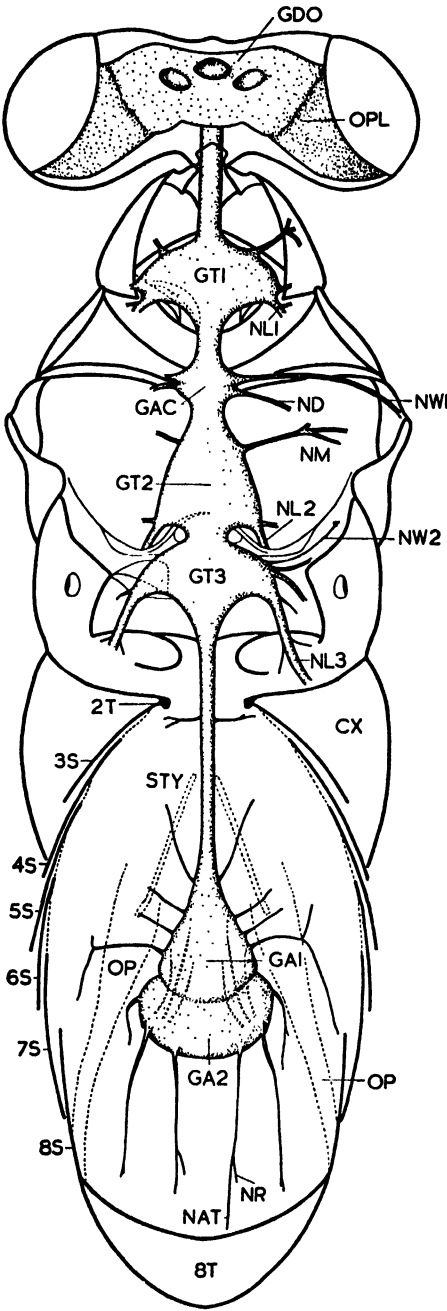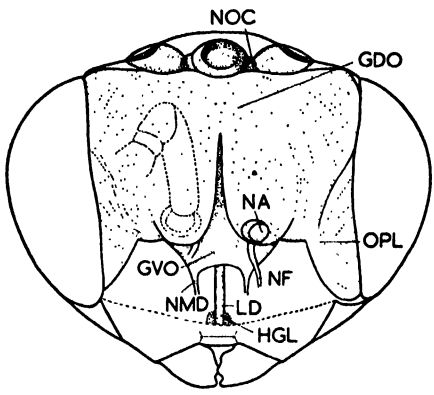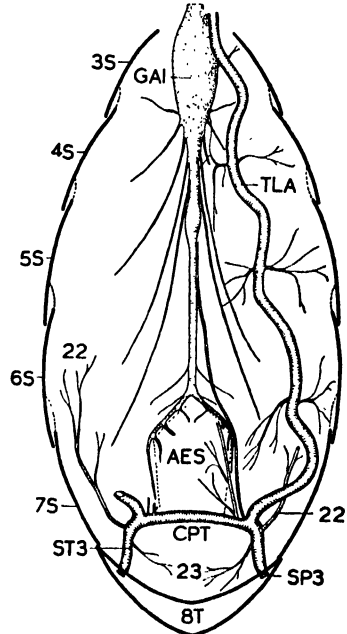
FIGS. 100 TO 102.
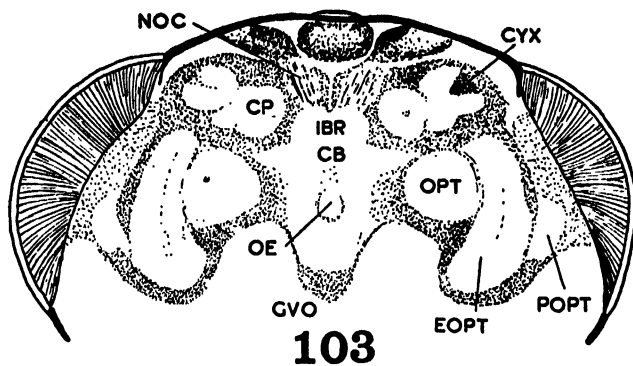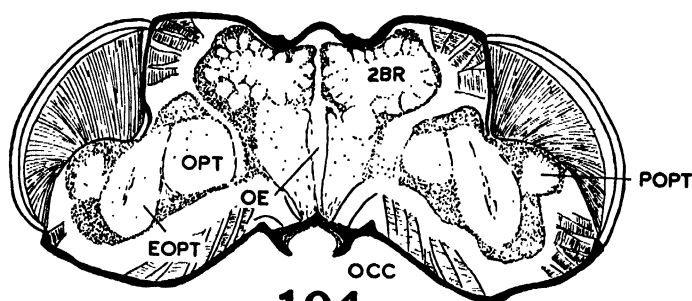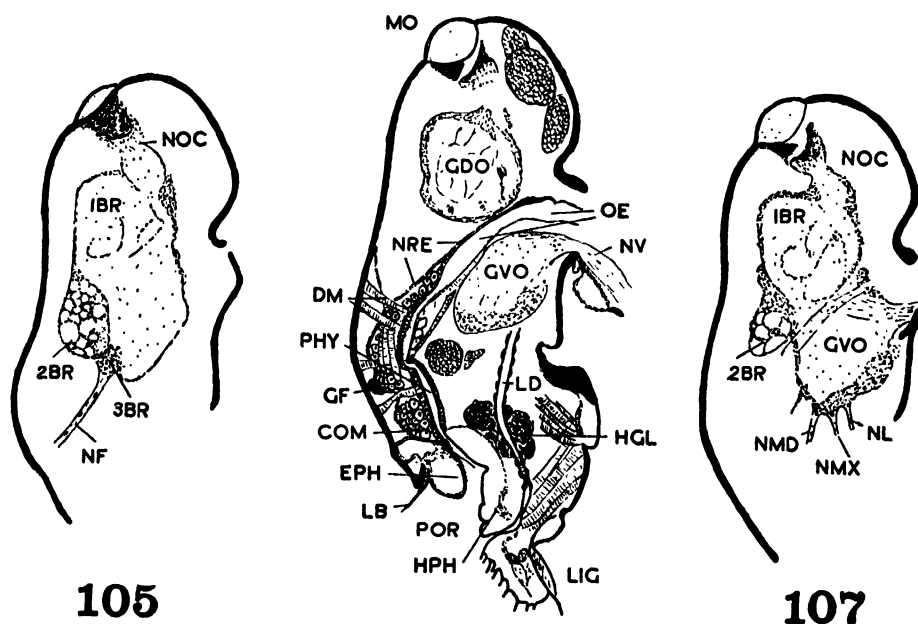
FIGS. 103 TO 107.

THE BIOLOGICAL ACTIVITY OF DDT AND RELATED COMPOUNDS[1]

By H. C. Browning,[2] F. C. Fraser,[3] S. K. Shapiro,[4] I. Glickman,[4] and M. Dubrûle[5]

Abstract

Sixty-eight compounds were examined for insecticidal activity, using *Drosophila melanogaster* as the test insect and the inner sides of glass containers as the test surface. The compounds included series of halogen analogues, reduced compounds, carbinols and their esters, ethanones, diphenylamines, and the gamma isomer of cyclohexane hexachloride. Activity was shown especially by the fluorine analogues and carbinol esters. Some compounds were briefly examined for mammalian toxicity.

The findings of other workers on the more important compounds are discussed and compared with those presently reported. No constant relation could be established between insecticidal activity and mammalian toxicity, or ease of dehydrochlorination. Low oil solubility was associated with low insecticidal potency (four compounds). No clear association of fat solubilizing or toxic properties with particular parts of the molecule was found.

The essentials of an active molecular structure are a two-carbon chain with one para-substituted phenyl ring on carbon 1 and a di- or tri-halogen group on a saturated carbon 2. Steric factors are also important.

Introduction

Subsequent to the discovery of the insecticidal properties of DDT, a number of investigations have been made of its isomers, analogues, and more distantly related compounds (3, 4, 5, 6, 7, 8, 10, 11, 19, 20, 22, 23, 27). Part of this work has been directed towards the finding of other insecticides, possibly with special properties differing from those of DDT, and part towards the elucidation of the relationship between chemical structure and potency.

From 1944 to 1946 a series of such compounds was synthesized in the Department of Chemistry, McGill University by Drs. D. L. Garmaise, G. T. Barry, and H. L. White (1, 25, 31) under the direction of Dr. R. Boyer. The present account deals with the testing of these substances together with some fluorine analogues (13) and diphenylamines supplied by Drs. S. Kirkwood and J. R. Dacey and a few compounds from industrial sources. This work was primarily a screening procedure, adapted for the rapid determination of insecticidal potentialities. Proverbs and Morrison (23) have given an account of more detailed tests of some of the same compounds using similar methods.

Methods

The test surface was the inside of a glass container on which a thin film of compound was left by evaporation from acetone. For each test a standard

solution was made containing 1 mgm. of pure DDT* per 100 cc. of acetone. The inner surfaces of half-pint milk bottles or of shell vials (7 by 2 cm.) were wet with the solution and dried at room temperature for approximately 20 hr. From 5 to 15 bottles or 50 to 100 vials were prepared. An identical series of test containers were made for each concentration of the compound under consideration. Usually three concentrations were tested initially: 2.5, 25, and 100 mgm. per 100 cc.

The test insects were adult *Drosophila melanogaster* four to six days after emergence. When vials were used as test containers 15 flies were placed in each of 50 to 100 vials; when bottles were used 100 flies were placed in each of 5 to 10 bottles. The two methods yielded results that did not differ significantly. The containers were stoppered with cotton wool plugs moistened with 5% aqueous molasses. They were incubated at 25° C. for 18 hr. when mortality counts were made. Each mortality percentage therefore refers to from 500 to 1500 flies scattered in from 5 to 100 separate groups. Control mortalities from containers treated with acetone alone were 2% or less.

In this way mortalities obtained using solutions of known concentrations of a given compound were compared with that obtained using a standard solution of pure DDT. The relative potency of a given compound was defined as the reciprocal of that concentration of the compound that produced approximately the same mortality as a 1 mgm. % solution of DDT under the conditions of the test. Compounds were often retested at different concentrations to obtain a mortality figure closer to that of the standard than was obtained in the initial test. They were also often retested as a check on the first results.

The test method (2) was a modification of that described by Morrison (18).

Results

The compounds that were tested are listed below. They are arranged according to structure rather than insecticidal potency. Those compounds that are discussed in later sections have been given a synonym. Insecticidal potencies relative to DDT have been inserted from Table I.

Table I lists the percentage mortalities for each test of each compound together with the figure for the corresponding standard. Compounds with an activity of 2/5ths to 1/10th of DDT were arbitrarily regarded as "good"; of 1/50th to 1/100th as "fair"; and of 1/250th to 1/500th as "poor". Compounds giving no mortality at a concentration of 500 mgm. % were considered to be totally inactive. At this concentration the film of compound on the glass of the test container is grossly visible and is considered equivalent to an unlimited supply of the substance.

* *M.p. 108 to 109° C.; thrice recrystallized from ethanol and 100% pure by chemophysical assay.*

TABLE I

PERCENTAGE MORTALITIES AMONG *Drosophila melanogaster* ON EXPOSURE TO DDT AND RELATED COMPOUNDS

Compound	No. of flies per test	DDT	Compounds									Relative potency
		1	2.5	5	10	25	50	75	100	250	500	
I. DDT	1500	14	53			61			77			
	750	12	54			72			89			1
	500	34	71			79			92			
	500	39	79			82			99			
III. Fluoro	750	14	13			100			100			2/5 to 1/5
	600	18	9	52								
IV. Bromo	500	41					7		19	24	40	1/500
VI. Trifluoro	750	14	—			—			21			
	500	52					15		52			1/100
VII. Tribromo	500	34	—			—			4			
	500	52									23	<1/500
VIII. Fluorotrifluoro	750	14	—			—			89			
	600	18					—	4				1/75 to 1/100
XI. Methyl	500	41	6	17	25	29			45			1/100
XV. Monochlor	1500	24*	4	4	12	25	27			44	48	
	1000	41			17							1/50 to 1/100
	500	52				31	41		56			
XIX.	500	38		4					46		92	>1/100
XX.	500	39					36		98		100	1/50
XXI. Carbinol	750	49*	—	—	4	—	26		80		100	
	500	39				12			91	99	100	
	500	34					35		98			1/50
	1000	39				—	33					
	500	36					25					
XXII. *l*-Carbinol	1000	33					72					
	500	36		—		19	71					1/25 to 1/50
XIII. *d*-Carbinol	1000	33					22					
	500	36					34		83			<1/50
XXIV. Fluor-carbinol	750	14	12			100			100			
	600	18	10	58								2/5 to 1/5
XXVI. Methoxy-carbinol	500	39					18			100	100	
	500	23					25	45				1/50
XXVII. Ethoxy-carbinol	500	38		6					98		99	
	500	74			7	16	27	82				1/50 to 1/75
XXVIII. Acetoxy-carbinol	500	34	9			53			76			
	1000	39	6	33								1/5 to 1/10
	500	52		18	66	70						

* 2.5 mgm. %.

TABLE I—*Concluded*

PERCENTAGE MORTALITIES AMONG *Drosophila melanogaster* ON EXPOSURE TO DDT AND RELATED COMPOUNDS—*Concluded*

Compound	No. of flies per test	Percentage mortalities with concentration in mgm. %										Relative potency
		DDT	Compounds									
		1	2.5	5	10	25	50	75	100	250	500	
XXXII. Butoxy-carbinol	500	24		—		—			16		29	1/100 to 1/500
XXXIX.	1000	45	—		—				—		99	
	500	51							17	53	98	1/250
XL.	1000	45	—			—			—		99	1/250 to 1/500
	500	51								24	99	
XLI.	500	24					45			100	100	>1/50
XLII.	500	24					44			99	100	>1/50
L.	500	40									35	
	500	57								21	28	<1/500
LI.	500	40									50	
	500	57				·				16	25	1/500
LVII.	500	38	—								44	1/500
LXIV.	500	24								13	18	<1/500
LXVII.	500	24								35	32	>1/250

A. ISOMERS

I. DDT: 1,1-*bis*(4-chlorophenyl)-2,2,2-trichloroethane

1
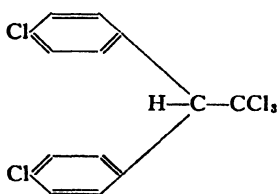
II. *o,p*-DDT: 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane

0
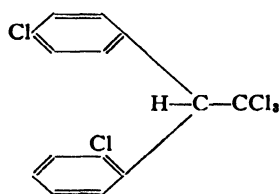
B. ANALOGUES

III. FLUORO-DDT: 1,1-*bis*(4-fluorophenyl)-2,2,2-trichloroethane

1–2/5
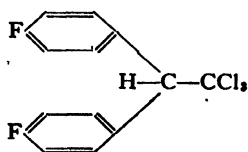
IV. BROMO-DDT: 1,1-*bis*(4-bromophenyl)-2,2,2-trichloroethane
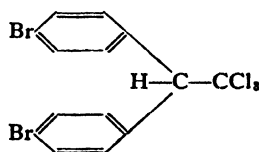
1/500

V. IODO-DDT: 1,1-*bis*(4-iodophenyl)-2,2,2-trichloroethane

0

I—⟨phenyl⟩
H—C—CCl₃
I—⟨phenyl⟩
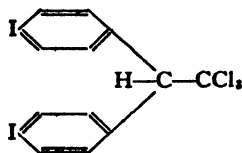
VI. TRIFLUORO-DDT: 1,1-*bis*(4-chlorophenyl)-2,2,2-trifluoroethane

1/100

Cl—⟨phenyl⟩
H—C—CF₃
Cl—⟨phenyl⟩
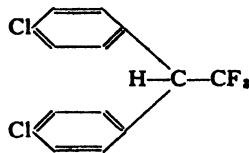
VII. TRIBROMO-DDT: 1,1-*bis*(4-chlorophenyl)-2,2,2-tribromoethane

<1/500

Cl—⟨phenyl⟩
H—C—CBr₃
Cl—⟨phenyl⟩
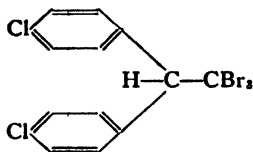
VIII. FLUOROTRIFLUORO-DDT: 1,1-*bis*(4-fluorophenyl)-2,2,2-trifluoroethane

>1/100

F—⟨phenyl⟩
H—C—CF₃
F—⟨phenyl⟩
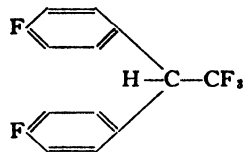
IX. 1,1-*bis*(4-Chlorophenyl)-1-fluoro-2,2-dichloro-2-fluoroethane

0

Cl—⟨phenyl⟩
F—C—CFCl₂
Cl—⟨phenyl⟩
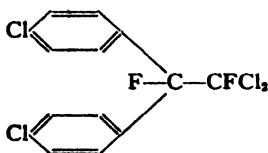
X. 1,1-*bis*(4-Chlorophenyl)-1-chloro-2,2,2-trichloroethane

0

Cl—⟨phenyl⟩
Cl—C—CCl₃
Cl—⟨phenyl⟩

XI. METHYL-DDT: 1,1-*bis*(4-methylphenyl)-2,2,2-trichloroethane

1/100

CH₃—⟨phenyl⟩
H—C—CCl₃
CH₃—⟨phenyl⟩
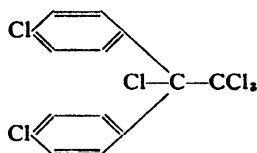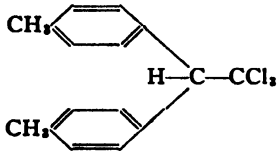
XII. 1,1-*bis*(4-Bromomethylphenyl)-2,2,2-trichloroethane

0

CH₂Br—⟨phenyl⟩
H—C—CCl₃
CH₂Br—⟨phenyl⟩
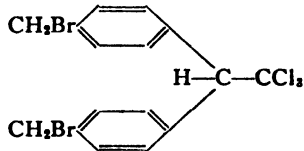
XIII. 1,1-*bis*(3-Methoxy-4-hydroxyphenyl)-2,2,2-trichloroethane

0

OCH₃
HO—⟨phenyl⟩
H—C—CCl₃
HO—⟨phenyl⟩
OCH₃
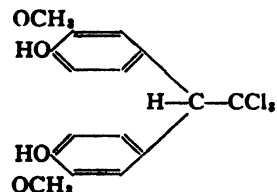
XIV. 1,1-*bis*(2-Nitro-4-chlorophenyl)-2,2,2,-trichloroethane

0

NO₂
Cl—⟨phenyl⟩
H—C—CCl₃
Cl—⟨phenyl⟩
NO₂
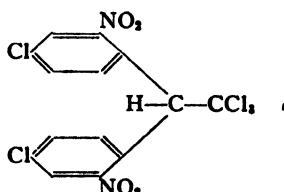
C. REDUCED COMPOUNDS

XV. MONOCHLOR-DDT: 1-phenyl-1-(4-chlorophenyl)-2,2,2-trichloroethane

>1/100

Cl—⟨benzene⟩

H—C—CCl₃

⟨benzene⟩
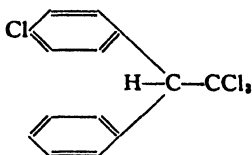
XVI. DT: 1,1-diphenyl-2,2,2-trichloroethane

0

⟨benzene⟩

H—C—CCl₃

⟨benzene⟩
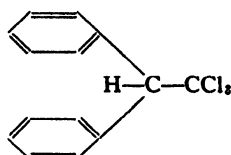
XVII. ETHYLENE-DDT: 1,1-*bis*(4-chlorophenyl)-2,2-dichloroethylene

0

Cl—⟨benzene⟩

C=CCl₂

Cl—⟨benzene⟩
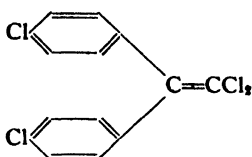
XVIII. BROMOETHYLENE-DDT: 1,1-*bis*(4-bromophenyl)-2,2-dichloroethylene

0

Br—⟨benzene⟩

C=CCl₂

Br—⟨benzene⟩
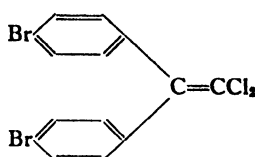
XIX. 1,1-*bis*(4-Chlorophenyl)-ethylene

>1/100

Cl—⟨benzene⟩

C=CH₂

Cl—⟨benzene⟩

XX. 1-(2-Chlorophenyl)-1-(4-chlorophenyl)-ethylene

1/50

Cl—⟨benzene⟩

C=CH₂

⟨benzene⟩—Cl
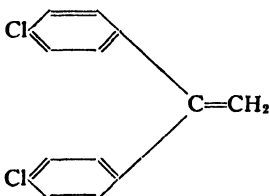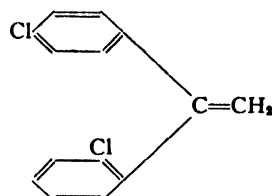
D. CARBINOLS, CARBINOL ANALOGUES, CARBINOL ESTERS

XXI. CARBINOL-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethanol

1/50

OH

Cl—⟨benzene⟩—C—CCl₃

H
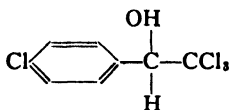
XXII. *levo*-1-(4-Chlorophenyl)-2,2,2-trichloroethanol

>1/50

XXIII. *dextro*-1-(4-Chlorophenyl)-2,2,2-trichloroethanol

<1/50

XXIV. 1-(4-Fluorophenyl)-2,2,2-trichloroethanol)

1–2/5

OH

F—⟨benzene⟩—C—CCl₃

H
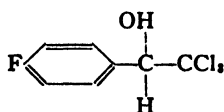
XXV. 1-(4-Trichloromethylphenyl)-2,2,2-trichloroethanol

0

OH

CCl₃—⟨benzene⟩—C—CCl₃

H
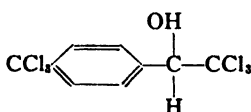
XXVI. 1-(4-Chlorophenyl)-1-methoxy-2,2,2-trichloroethane

1/50
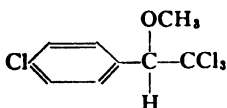
XXVII. 1-(4-Chlorophenyl)-1-ethoxy-2,2,2-trichloroethane

>1/75
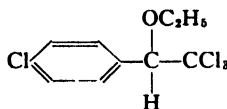
XXVIII. Acetoxy-carbinol-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethyl acetate

>1/10
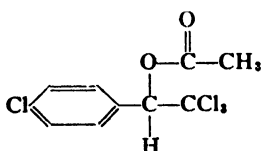
XXIX. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl trichloroacetate

0
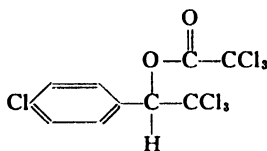
XXX. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl monochloroacetate

0
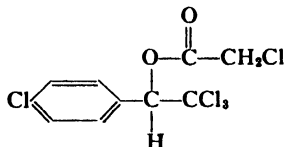
XXXI. Propanoxy-carbinol-DDT: 1-(4-chlorophenyl)-2,2,2-trichloroethyl propionate

0
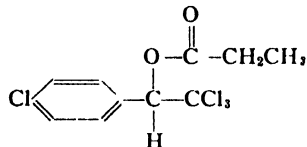
XXXII. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl butyrate

>1/500
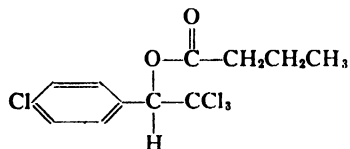
XXXIII. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl acetoxypropionate

0
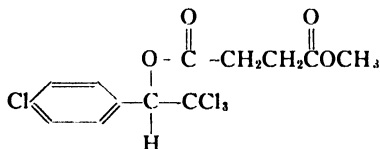
XXXIV. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl benzoate

0
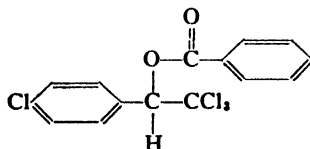
XXXV. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl benzamide

0
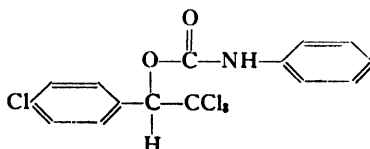
XXXVI. 1-(4-Chlorophenyl)-2,2,2-trichloroethyl 4-chlorobenzoate
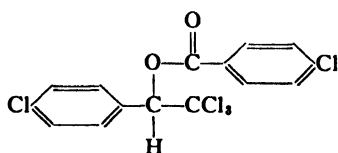
XXXVII. Tri-(4-chlorophenyl)-methanol

0
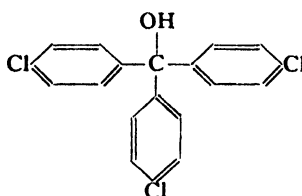
0

XXXVIII. 1-Butyl-2,2,2-trichloroethanol

0

$$C_4H_9O-\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}-CCl_3$$
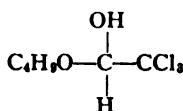
E. ETHANONES, ALDEHYDES, BENZOPHENONES, SULPHONES

XXXIX. 1-(4-Chlorophenyl)-2,2,2-trichloroethanone
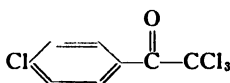
XL. 1-(4-Chlorophenyl)-2,2-dichloroethanone

1/250
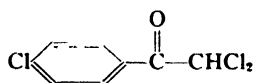
>1/500

XLI. 1-(4-Trichloromethylphenyl)-2,2,2-trichloroethanone
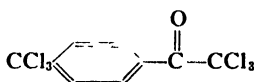
XLII. 1-(4-Trichloromethylphenyl)-2,2-dichloroethanone

> 1/50
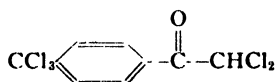
> 1/50

XLIII. 1-(4-Chlorophenyl)-ethanone
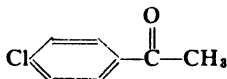
XLIV. 4-Chlorobenzaldehyde

0
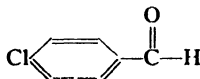
0

XLV. BENZOPHENONE: 4,4'-dichlorobenzophenone
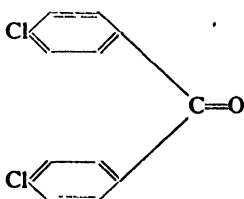
XLVI. *o,p*-BENZOPHENONE: 2,4'-dichlorobenzophenone

0
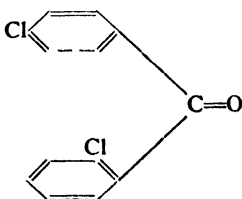
0

XLVII. Sulphone: 4,4'-dichlorophenyl sulphone
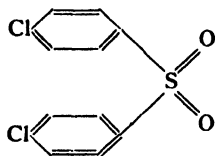
0

XLVIII. 4,4'-Dibromophenyl sulphone
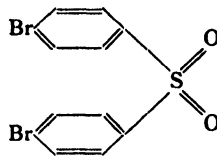
0

F. DIPHENYLAMINES

XLIX. N-Trichloromethyl-4,4'-dichloro-phenylamine
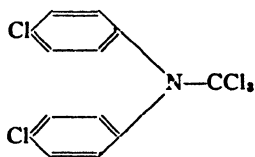
0

L. N-Methyl-4,4'-dichlorophenylamine
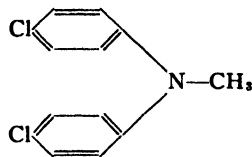
<1/500

LI. 4,4'-Dichlorophenylamine
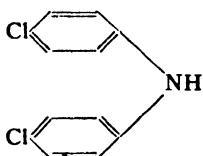
1/500

LII. N-Trichloroacetyl-4,4'-dichlorophenyl-amine
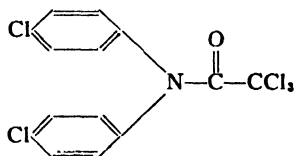
0

LIII. N-Trichloroacetyldiphenylamine
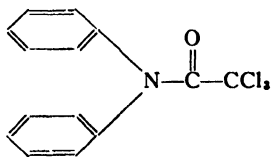
0

LIV. 4,4'-Dinitrophenylamine
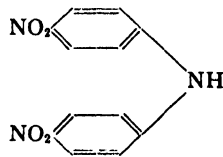
0

G. MISCELLANEOUS COMPOUNDS

0

LV. Hexachloroethane
$CCl_3 . CCl_3$

0

LVI. Pentachloroethane
$CCl_3 . CCl_2 . H$

LVII. 1-Butoxy-1-chloro-2,2,2-trichloroethane

1/500

$C_4H_9O—CH . Cl . CCl_3$

LVIII. Chloral hydrate

0

$CH(OH)_2 . CCl_3$
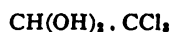
LIX. 4,4'-Dichlorodibenzyl disulphide

0

$$Cl{-}\langle\ \rangle{-}CH_2{-}S$$
$$Cl{-}\langle\ \rangle{-}CH_2{-}S$$
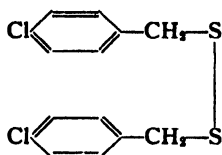
LX. 2,2'-Dichlorodibenzyl disulphide

0

$$\langle\ \rangle{-}CH_2{-}S$$
$$Cl$$
$$Cl$$
$$\langle\ \rangle{-}CH_2{-}S$$
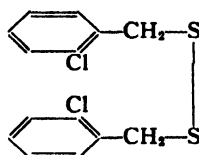
LXI. 3,4,3',4'-Tetrachlorodibenzyl disulphide

0

$$Cl{-}\langle\ \rangle{-}CH_2{-}S$$
$$Cl$$
$$Cl$$
$$Cl{-}\langle\ \rangle{-}CH_2{-}S$$
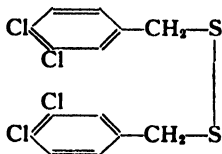
LXII. 2,4,2',4'-Tetrachlorodibenzyl disulphide

0

$$Cl{-}\langle\ \rangle{-}CH_2{-}S$$
$$Cl$$
$$Cl$$
$$Cl{-}\langle\ \rangle{-}CH_2{-}S$$
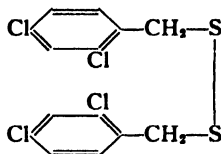
LXIII. 1,1-*bis*(2,5-Dichlorothienyl)-2,2,2-trichloroethane

0

$$H{-}C{-}CCl_3$$
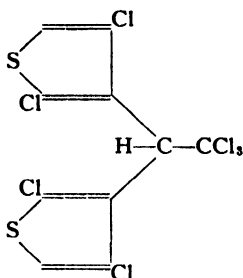
LXIV. 4-Bromobenzenesulphonic acid

<1/500

$$Br{-}\langle\ \rangle{-}SO_3H$$
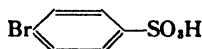
LXV. 1,1-*bis*(2-Chlorophenyl)-acetic acid

0

$$H{-}C{-}C{-}OH$$
$$O$$
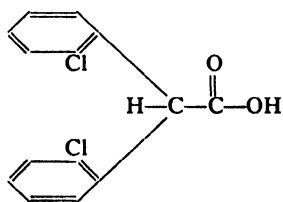
LXVI. 4,4'-Dichlorodiphenylbenzil

0

$$Cl{-}\langle\ \rangle{-}C{=}O$$
$$Cl{-}\langle\ \rangle{-}C{=}O$$
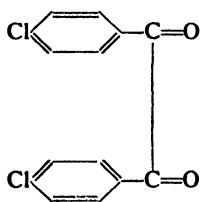
LXVII. 1,4-Dichlorobenzene

>1/250

$$Cl{-}\langle\ \rangle{-}Cl$$
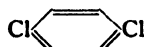
LXVIII. Gamhexane: γ,1,2,3,4,5,6-hexachlorocyclohexane
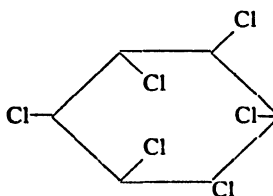
5

The standard 1 mgm. % of DDT gave a mortality varying on different occasions from 12 to 74%. Ideally comparisons should be made between concentrations of DDT and of the compounds that give a 50% mortality, i.e. the LD_{50}. However, when the mortality for a standard of 1 mgm. % was about 15%, the 50% mortality level would lie around 2.5 mgm. %; and when about 38% at approximately 1.5 mgm. % (Table I, DDT). Thus the factor of discrepancy between the relative potencies expressed in Table I and those that would have been obtained from the 50% mortality level is relatively small unless the dosage–mortality curves for DDT and the compound in question are very different in slope.

Morrison (17) says that the results of comparative insecticidal tests are largely a function of the methods used. In Table I two figures are shown that illustrate this contention. In the first test of monochlor-DDT (XV) impregnated paper rectangles were used as the test surface (18), instead of the glass of the test containers, and the standard concentration of DDT was 2.5 mgm. % instead of 1 mgm. %. By this method the relative potency of monochlor-DDT was 1/10th of DDT rather than the figure of 1/50 to 1/100th derived from later tests using the usual technique. Again, in the first test of carbinol-DDT the standard concentration of DDT was 2.5 mgm. % instead of 1 mgm. %. The relative potency was 1/30th rather than 1/50th. However, in view of the great variability between tests when the same technique was used, it is impossible to state how much of the difference between the results of the two methods is due to the difference in technique and how much is due to random variation.

Tests of gamhexane (hexachlorocyclohexane—Compound LXVIII) included very low concentrations and the results are expressed in Table II. The relative potency was approximately 5.

TABLE II

PERCENTAGE MORTALITIES AMONG *Drosophila melanogaster* ON EXPOSURE TO DDT AND GAMHEXANE

No. of flies per test	DDT	Gamhexane									
	1	0.1	0.2	0.25	0.4	0.5	0.6	1	2.5	25	100
500	39							75	91	100	100
1000	39	11		47		81		82			
1000	22	8	18								
1000	24		5		32						
1000	41			27	69		77				
1500	35			43							

A comparison was made of the speeds of action of gamhexane, carbinol-DDT, and DDT. Tests were set up in the usual manner but mortality counts were made at the end of six hours as well as after the usual 18 hr. The

figures in Table III show that both gamhexane and carbinol-DDT cause death far more rapidly than DDT at concentrations that produce comparable mortalities after 18 hr. (DDT 2.5 mgm. %; carbinol-DDT 100 mgm. %; gamhexane 1 mgm. %.)

TABLE III

PERCENTAGE MORTALITIES AMONG 500 *Drosophila melanogaster* ON EXPOSURE TO DDT, CARBINOL-DDT, AND GAMHEXANE FOR 6 AND 18 HR.

Compound	Percentage mortalities with conc. in mgm. %						Hours of exposure
	1	2 5	25	100	250	500	
I. DDT	2	6	7	63			6
XXI. Carbinol				63	90	97	6
LXVIII. Gamhexane	60	90	100	100			6
I. DDT	39	79	82	99			18
XXI. Carbinol				91	99	100	18
LXVIII. Gamhexane	75	91	100	100			18

A number of compounds (*o,p*-DDT, trifluoro-DDT, methyl-DDT, carbinol-DDT, acetoxy-carbinol-DDT, propanoxy-carbinol-DDT, and 1-(4-chlorophenyl)-2,2-dichloroethanone) were tested for synergism. A solution of 0.2 mgm. of compound and 0.6 mgm. of DDT in 100 cc. of acetone was used for the preparation of test containers. The percentage mortalities after 18 hr. exposure of 1000 flies were compared to those from 0.6, 0.75, and 0.9 mgm. % of pure DDT. No synergism was found.

The same type of test was made with a mixture of 0.1 mgm. % of gamhexane and 0.6 mgm. % of DDT. The percentage mortalities were compared with those produced by the same compounds used independently. Separately gamhexane and DDT gave 15 and 22% mortality respectively; as a mixture they gave 50% mortality. Further tests would be necessary to decide whether this indicates a synergistic effect or merely a random variation.

Mammalian Toxicity

Preliminary tests for mammalian toxicity were done on a number of compounds. Each was dissolved in a minimum amount of olive oil and administered by forced feeding to four mice. The average weight of the animals was 25 gm. As an initial dose, 5 mgm. of compound was given, since neurotoxic symptoms were induced in all animals by DDT at this level. On five subsequent days 10 mgm. was given. The animals were observed through the period of treatment and for five days thereafter.

Table IV shows the exhibition of neurotoxic symptoms (tremors and hyperexcitability) and an arbitrary classification of toxicity; "high" indicates that three or four mice died; "medium" that one or two mice died; and "low" that none died. In addition to those compounds listed in the table groups of four mice received 25 mgm. only of acetoxy-carbinol-DDT and of sulphone; and

TABLE IV

INSECTICIDAL POTENCIES AND MAMMALIAN TOXICITIES OF DDT AND RELATED COMPOUNDS

Compound		Relative insecticidal potency	Mammalian toxicity	Neurotoxic symptoms
I.	DDT	1	High	XX
II.	o,p-DDT	0	Low	—
III.	Fluoro	1/2.5 – 1/5	High	XX
IV.	Bromo	1/500	High	XX
V.	Iodo	0	Medium	XX
VII.	Tribromo	0	Low	—
VIII.	Fluorotrifluoro	1/75 – 1/100	Low	—
IX.		0	Medium	—
X.		0	High	—
XI.	Methyl	1/100	Low	—
XII.	Bromomethyl	0	Low	—
XV.	Monochlor	1/50 – 1/100	Medium	X
XVI.	DT	0	Low	—
XVII.	Ethylene	0	Medium	—
XXI.	Carbinol	1/50	Medium	—
XXXI.	Propanoxy-carbinol	0	Low	—
XL.		1/250 – 1/500	Low	—
XLV.	Benzophenone	0	Low	—
XLVI.	o,p-benzophenone	0	Low	—
LXVII.		0	Low	—
LXVIII.	Gamhexane	5	High	—

XX = *tremors and hyperexcitability in all animals.*
X = *faint tremors and mild hyperexcitability in one animal.*

15 mgm. only of trifluoro-DDT and of bromoethylene-DDT. None of these compounds produced neurotoxic symptoms but the last named killed two of the animals.

Discussion

Relative Insecticidal Potency

In Table V are grouped the more interesting compounds with the findings from the present work and that of other authors. Two compounds, DDD (1,1-*bis*(4-chlorophenyl)-2,2-dichloroethane), and methoxy-DDT (1,1-*bis*(4-methoxyphenyl)-2,2,2-trichloroethane), are included although not the subject of the present work. Where available a figure for potency relative to that of DDT has been inserted; the comparative ratings are always relative to the entire findings of the author.

The methods of testing differed. For instance Busvine (3, 4, 5) usually applied the compounds in an oily spray, which may have facilitated penetration of the insect cuticle. Haller (11) and Prill (22) are usually referring to aquatic insect larvae where ingestion of the compound can occur. However, despite variations in the technique of testing and estimation there is good general agreement.

TABLE V

INSECTICIDAL POTENCIES OF VARIOUS COMPOUNDS RELATIVE TO DDT AS DERIVED FROM VARIOUS AUTHORS. (REFERENCE AND INSECT SPECIES:— *Br.*, PRESENT REPORT, *Drosophila; Ps.*, (23), *Drosophila; Bs.*, (3, 4, 5), *Cimex, Pediculus; Hr.*, (11), *Anopheles; Pr.*, (22), *Culex; Sr.*, (27), *Carpocapsa; Dz.*, (8), *Pediculus, Calliphora, Formica, Calandra, Ephestia, Tineola; Lr.*, (15), *Tineola, Anthrenus, Attagenus; Mr.*, (19), *Calliphora, Formica, Calandra, Trogium, Thaumetopoea; Ms.*, (20), *Calliphora, Tineola.*)

Compound	Br.	Ps.	Bs.	Hr.	Pr.	Sr.	Ds.	Lr.	Mr.	Ms.
I. DDT	Good 1	Good 1	Good 1	Good 1	Good 1	Good	Good	Good	Good	Good
II. o,p-DDT	Nil 0	Poor 1/145	Poor 1/20–1/28	Nil 0			Poor		Fair	
III. Fluoro	Good 1/5–2/5	Good 9	Fair 1/3–1/10	Fair 1/2	Good 1		Good		Good	
IV. Bromo	Poor 1/500	Fair 1/26	Good 1/2–1/3	Good 1	Fair 1/5	Good		Fair	Fair	
V. Iodo	Nil 0	Nil 0			Fair 1/5					
VII. Tribromo	Poor 1/500			Poor 1/10				Good		Good
XI. Methyl	Fair 1/100	Fair 1/10	Fair 1/6–1/7	Fair 1/2	Good 1	Good	Fair		Good	
XV. Monochlor	Fair 1/100	Poor 1/90	Fair 1/8	Fair 1/2	Fair 1/5				Good	
XVI. DT	Nil 0	Nil 0	Poor 1/25	Nil 0	Poor 1/10–1/20	Poor	Poor		Fair	
XVII. Ethylene	Nil 0		Poor 1/20–1/30		Fair 1/5	Nil	Poor		Fair	Poor
XXI. Carbinol	Fair 1/50	Fair 1/25	Poor 1/20				Good			
DDD		Fair 1/17	Good 1/2–1/6	Good 1			Good	Fair	Good	Good
Methoxy			Good 1/2–1/3	Good 1	Good ' 1			Good	Good	
LXVIII. Gamhexane	Good 5	Good 20	Good 18–20	Good 9						

The ortho-para isomer of DDT was totally inactive in the present tests although some workers ascribe "poor" action to it. This compound was tested both after repeated recrystallization from organic solvents and after synthesis from orthocarbinol-DDT and chlorobenzene to eliminate contamination with DDT itself (25). Such contamination could be responsible for the above-mentioned activity. This isomer has been reported as the chief impurity in technical DDT (9, 12).

Fluoro-DDT has been found to be active by all workers but there is a big range in the comparative potencies expressed. Busvine (4) and Haller (11) both give it as less effective than the bromine analogue. Proverbs and Morrison (23) call attention to its volatility and this property may account for differing estimates. Bromo-DDT is listed as equitoxic with DDT by Haller but the figure is derived from comparisons where the weakest concentration of DDT already produced a 100% mortality.

Carbinol-DDT was rated as "fair" in the present work and by Proverbs and Morrison (23), but as poor by Busvine (4). Domenjoz (8) found it as active as DDT against the louse, fly, weevil, and ant but inactive against the clothes moth larva. Present tests included the optically active forms (31). *Levo*-carbinol was more active and *dextro*-carbinol less active than the racemic mixture (Compounds XXII and XXIII, Table I).

Carbinol-DDT was especially interesting in that it acted more rapidly than DDT and appeared to have a different mode of action. The dosage/mortality-probit line was appreciably steeper than that of DDT. Poisoned fruit flies did not show convulsive movements but died as though narcotized. The dead insects had their wings folded in the normal horizontal position and did not appear desiccated in contrast to the flies killed by DDT, which had vertically outstretched wings and shrivelled bodies. So closely did the dead insects resemble those that were merely anesthetized with ether that, in one test, they were removed from the test containers and kept under observation. They did not recover.

Carbinol-DDT is also interesting on account of its chemophysical properties. Despite the replacement of one para-substituted phenyl ring by a hydroxyl group it has an appreciable potency relative to that obtained through loss of the para substituent (monochlor-DDT). It has a low melting point and a water-solubilizing hydroxyl group (although Domenjoz (8) found it to be nearly three times as soluble in olive oil as DDT.) The first of a series of esters, acetoxy-carbinol-DDT, showed a sharp rise in potency to 1/10th that of DDT and its mode of action seemed to be the same as that of the parent carbinol. Replacement of the hydroxyl group with methoxy and ethoxy groups (Compounds XXVI and XXVII) produced substances with "fair" activity although slightly less potent than the parent compound. Replacement of the para chlorine by fluorine gave a highly active compound, paralleling the same replacement in DDT itself. The activity of derivatives with one or two trichloromethyl groups on the first carbon atom might be interesting:—
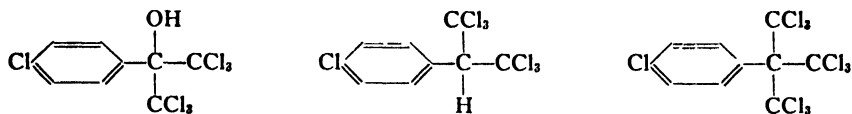
Chemical Structure and Toxicity

Of the compounds tested, 16 had relative potencies of from 1/100th to 2/5ths that of DDT (Table I). The basic structure of their molecules was a two-carbon chain with a para-substituted phenyl ring on carbon 1 and a di- or

tri-halogen group on a saturated carbon 2. The only exceptions were the chlorophenylethylenes (Compounds XIX and XX) where the carbon chain was unsaturated.
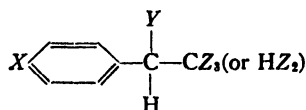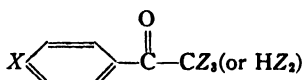
This basic structure is represented by the above skeleton formulae where X may be F, Cl, CH_3, or (sometimes) CCl_3; where Y may be C_6H_5Cl, C_6H_6, OH, OCH_3, OC_2H_5, or $O . CO . CH_3$; and where Z may be F or Cl. These findings are similar to those of von Oettingen and Sharpless (21) and of Smith, Bauer, Stohlman, and Lillie (29) for mammalian toxicities but there are well marked exceptions.

Table VI shows the simplified findings available from four authors for the olive oil solubility, ease of dehydrochlorination, and mammalian toxicity of a series of the compounds. Insecticidal (and mammalian) toxicities have been inserted from the present work, where possible, to give values uncomplicated by other than unaided contact action.

The table shows that compounds with high insecticidal activity give neurotoxic symptoms in mammals but do not necessarily have high mammalian toxicity (DDT, fluor-DDT, methoxy-DDT, DDD—the latter is a fair insecticide only). Conversely compounds with high mammalian toxicity are not necessarily insecticidally active (bromo- and iodo-DDT). o,p-DDT, tri-bromo-DDT, DT, ethylene-DDT, and sulphone have both low mammalian and insecticidal toxicity. Carbinol-DDT, methyl-DDT, and monochlor-DDT have fair insecticidal activity and low mammalian toxicity, producing no neurotoxic symptoms.

These correlations are thought by some of us (H.C.B., M.D.) to suggest that the mechanism of toxic action may be similar for insect and mammal and that the differences are due to the manner of entry into the tissues. Savit, Kollros, and Tobias (26, 30) showed that the LD_{50} to insects for both DDT and gamhexane was of the same order whether administered externally or internally. This is in complete contrast with the conditions in mammals where unaided percutaneous absorption is negligible. In insects the effective compounds must have an ability to penetrate the integument, a layer designed to hinder the passage of substances in either direction. From the integument it may pass into the hypodermis and thence to the hemolymph or (24) enter the lipoid nerve sheath directly.

Laüger, Martin, and Müller (15) suggested that fat solubility of contact insecticides promoted entry. Martin and Wain (16) made the same assumption, adding a suggestion that toxic action itself was due to the liberation of hydrochloric acid at the vital centers. In Table VI four compounds are less fat soluble than DDT; bromo-DDT, iodo-DDT, tribromo-DDT, and sulphone. The first two are highly toxic to mammals but inert insecticidally; the last two are low in toxicity to both groups of animals. Conversely it

TABLE VI

INSECTICIDAL TOXICITY, OLIVE OIL SOLUBILITY, EASE OF DEHYDROCHLORINATION, AND MAMMALIAN TOXICITY OF VARIOUS COMPOUNDS RELATED TO DDT

(O., VON OETTINGEN AND SHARPLESS (21); D., DOMENJOZ (8); B., BUSVINE (3, 4, 5); M., MÜLLER (19); Br., PRESENT WORK)

Compound	Insect toxicity	Olive oil solubility			Ease of dehydrochlorination			Mammalian toxicity				Neurotoxic symptoms			
	Br.	O.	D.	B.	O.	D.	M.	O.	D.	S.	Br.	O.	D.	S.	Br.
I. DDT	Good	1.0	1.0	1.0	1.0	1.0	1.0	1	1	1	High	XX	XX	XX	XX
II. o,p-DDT	Nil	>4.0	1.1	2.5	0.2	0.1	0.1	>5	8		Low	—	—		—
III. Fluoro	Good	0.2	>4.0		0.2	0.6	1.0	5	1	1	High	X	XX		XX
IV. Bromo	Poor	0.0			1.5		1.0	1			High	XX			XX
V. Iodo	Nil	0.2			1.5			2			Med.	XX		XX	XX
VII. Tribromo	Poor				10.0			4			Low	—			—
XI. Methyl	Fair	1.2	0.8	2.0	0.0	0.0	0.3	>3	8		Low	—	—		—
XV. Monochlor	Fair	2.8	2.7	3.0	0.2			>5			Med.	—			—
XVI. DT	Nil	3.4	0.9		0.2	0.1	0.7	>4	5	14	Low	—	XX	—	—
XVII. Ethylene.	Nil	2.0		2.0	0.0	0.0		>3	3	7	Med.	—	—	X	
XXI. Carbinol	Fair		2.8						8		Med.		—		
XLVII. Sulphone	Nil		0.1		0.0	0.0			60				—		
DDD	Fair*	0.8	0.8	1.0	0.3	0.8	1.0	>5	15	20		—	XX	X	
Methoxy	Good**	1.0	0.5	1.0	0.0	0.0	0.3	>4	5	50		—	XX	—	

* *Proverbs and Morrison (23).*
** *Table V.*
NOTE: X = *mild or slight tremors.*
　　　 XX = *"DDT" tremors.*

might be expected that some of the compounds with low mammalian toxicity are fair insecticides owing to compensatory high fat solubility (monochlor-DDT, carbinol-DDT, methoxy-DDT, and DDD). None of the six compounds showing fair or greater insecticidal activity had a low oil solubility. Solubilities of the compounds in substances more closely related to the lipids of insect integument than a vegetable oil might be more revealing. No correlation is seen between ease of dehydrochlorination and toxicity.

Laüger, Martin, and Müller (15) considered that the trihalogen group was fat-solubilizing and the para-chlorophenyl rings toxic. Martin and Wain (16) offered the reverse concept. Kirkwood and Phillips (14), with evidence from fluoro-DDT and trifluoro-DDT, supported the first theory. Tribromo-DDT has a very low fat solubility (21). Conversely, the halogen para phenyl substituents decrease fat solubility in the order fluorine, chlorine, bromine, and iodine.

This attribution of specific properties to specific parts of the molecule is unjustified. The removal of substituents (monochlor-DDT and DT) or even the removal of an entire chlorophenyl ring (carbinol-DDT) increases fat solubility. When considering the reactions of functional groups, the modifying influences of the rest of the molecule should not be forgotten. This is especially important in biological systems. The replacement of a single hydrogen atom by chlorine (Compound X), without affecting either the chlorophenyl or tri-chlorethane groups abolishes all activity. Conspicuous among all compounds is the ortho-para isomer of DDT. Merely by a shift in position of one chlorine atom all insecticidal action is lost and mammalian toxicity much reduced. The 2,2'-isomer has also been found inactive (6, 19) but the 3,4'-isomer was a good insecticide (7, 19). Similar findings are reported for the isomers of hexachlorocyclohexane of which gamhexane is but one (28). Internal energy relations of components of a molecule and allied steric factors are equal in importance with the actual nature of the constituent atoms.

Acknowledgment

References

1. BARRY, G. T. Synthesis of DDT homologs. In preparation.
2. BROWNING, H. C., FRASER, F. C., SHAPIRO, S. K., GLICKMAN, I., and DUBRÛLE, M. The biological assay of DDT (1,1-*bis*(4-chlorophenyl)-2,2,2-trichloroethane). In preparation.
3. BUSVINE, J. R. Insecticidal action of DDT. Nature, 156 : 169-170. 1945.
4. BUSVINE, J. R. Insecticidal tests with analogues of DDT. J. Soc. Chem. Ind. 65 : 356-360. 1946.
5. BUSVINE, J. R. New synthetic contact insecticides. Nature, 158 : 22. 1946.
6. CRISTOL, S. J., HALLER, H. L., and LINDQUIST, A. W. Toxicity of DDT isomers to some insects affecting man. Science, 104: 343-344. 1946.
7. DEONIER, C. C. and JONES, H. A. TDE, 1,1-dichloro-2,2-*bis*-(*p*-chlorophenyl)-ethane, as an anopheline larvicide. Science, 103 : 13-14. 1946.

8. DOMENJOZ, R. Action biologique de quelques dérivés du DDT. Helv. Chim. Acta, 29 : 1317-1322. 1946.

9. FORREST, J., STEPHENSON, O., and WATERS, W. A. Chemical investigations of the insecticide "DDT" and its analogues. Part I. Reactions of "DDT" and associated compounds. J. Chem. Soc. : 333-339. 1946.

10. GINSBURG, J. M. Comparative toxicity of DDT isomers and related compounds to mosquito larvae and fish. Science, 105 : 233-234. 1947.

11. HALLER, H. L. Wartime development of insecticides. Ind. Eng. Chem. 39 : 467-473. 1947.

12. HALLER, H. L., BARTLETT, P. D., DRAKE, N. L., NEWMAN, M. S., CRISTOL, S. J., EAKER, C. M., HAYES, R. A., KILMER, G. W., MAGERHEIN, B., MUELLER, G. P., SCHNEIDER, A., and WHEATLEY, W. The chemical composition of technical DDT. J. Am. Chem. Soc. 67 : 1591-1602. 1945.

13. KIRKWOOD, S. and DACEY, J. R. Synthesis of some analogues of 1,1-*bis*(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT). I. Three fluorine analogues. Can. J. Research, B, 24 : 69-72. 1946.

14. KIRKWOOD, S. and PHILLIPS, P. H. The relationship between the lipoid affinity and the insecticidal action of 1,1-*bis*(*p*-chlorophenyl)-2,2,2-trichloroethane and related substances. J. Pharmacol. 87 : 375-381. 1946.

15. LÄUGER, P., MARTIN, H., and MÜLLER, P. Über Konstitution und toxische Wirkung von natürlichen und neuen synthetischen insektentötenden Stoffen. Helv. Chim. Acta, 27 : 892-928. 1944.

16. MARTIN, H. and WAIN, R. L. Insecticidal action of DDT. Nature, 154 : 512-513. 1944.

17. MORRISON, F. O. The standardizing of a laboratory method for comparing the toxicity of contact insecticides. Can. J. Research, D, 21 : 35-75. 1943.

18. MORRISON, F. O. Comparing the toxicity of synthetic organic compounds. Ann. Rept. Entomol. Soc. Ontario : 18-20. 1946.

19. MÜLLER, P. Über Zusammenhänge zwischen Konstitution und insektizider Wirkung I. Helv. Chim. Acta, 29 : 1560-1580. 1946.

20. MYLIUS, A. and KOECHLIN, H. Berichtigungen zur Arbeit von P. Läuger, H. Martin, und P. Müller "Über die Konstitution und toxische Wirkung von natürlichen und neuen synthetischen insektentötenden Stoffen". Helv. Chim. Acta, 29 : 405-411. 1946.

21. OETTINGEN, W. F. VON and SHARPLESS, N. E. The toxicity and toxic manifestations of 2,2,*bis*-(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) as influenced by chemical changes in the molecule. J. Pharmacol. 88 : 400-413. 1946.

22. PRILL, E. A., SYNERHOLM, M. E., and HARTZELL, A. Some compounds related to the insecticide "DDT" and their effectiveness against mosquito larvae and houseflies. Contr. Boyce Thompson Inst. 14 : 341-353. 1946.

23. PROVERBS, M. D. and MORRISON, F. O. The relative insecticidal activities of DDT and related organic molecules. Can. J. Research, D, 25 : 12-44. 1947.

24. RICHARDS, A. G. Lipoid nerve sheaths in insects and their probable relation to insecticidal action. J. New York Entomol. Soc. 51 : 55-69. 1943.

25. ROSS, J. H., BOYER, R., GARMAISE, D. L., BARRY, G. T., and WHITE, H. L. Progress reports to the National Research Council, Canada. Unpublished. 1944–1946.

26. SAVIT, J., KOLLROS, J. J., and TOBIAS, J. M. Measured dose of gamma hexachlorocyclohexane (γ666) required to kill flies and cockroaches and a comparison with DDT. Proc. Soc. Exptl. Biol. Med. 62 : 44-48. 1946.

27. SIEGLER, E. H. and GERTLER, S. I. Toxicity of diaryl trichloroethanes and dichloroethylenes to codling moth. J. Econ. Entomol. 37 : 845. 1944.

28. SLADE, R. E. The γ-isomer of hexachlorocyclohexane (gammexane). Chem. Ind. 314-319. 1945.

29. SMITH, M. I., BAUER, H., STOHLMAN, E. F., and LILLIE, R. D. The pharmacologic action of certain analogues and derivatives of DDT. J. Pharmacol. 88 : 359-365. 1946.

30. TOBIAS, J. M., KOLLROS, J. J., and SAVIT, J. Relation of absorbability to the comparative toxicity of DDT for insects and mammals. J. Pharmacol. 86 : 287-293. 1946.

31. WHITE, H. L. Synthesis of DDT homologs. In preparation.

THE INSECTICIDAL ACTIVITY OF DDT AND RELATED COMPOUNDS AGAINST DIFFERENT INSECT SPECIES[1]

By H. C. Browning,[2] S. K. Shapiro,[3] and M. Dubrûle[4]

Abstract

DDT and its fluoro- and methyl-analogues, its carbinol and carbinol acetate, and monochlor-DDT (1-(4-chlorophenyl)-1-phenyl-2,2,2-trichloroethane) were tested for contact insecticidal activity against two beetles (*Calandra granaria* and *Tribolium confusum*), a moth (*Ephestia kuhniella*), a plant bug (*Oncopeltus fasciatus*), and a cockroach (*Blatella germanica*).

Fluoro-DDT showed specificity against the cockroach and carbinol-DDT against the plant bug. Comparisons with the results of earlier testing against *Drosophila melanogaster* justified its use as an indicator species.

Introduction

A large series of compounds related to DDT had been tested against *Drosophila melanogaster* (2) in co-ordination with a program of synthesis in the Department of Chemistry, McGill University (1, 14, 19) and the National Research Council Laboratories (9). At the conclusion of this program it was felt desirable to examine selected compounds for potency against other insects, partly to detect specific activities and partly to examine the validity of using *Drosophila melanogaster* as an indicator of insecticidal efficiency.

In the previous work compounds were arbitrarily classified as having good activity if the relative potency (compared to DDT) was 1/10th or more; as fair if from 1/50th to 1/100th; and as poor if from 1/250th to 1/500th. Five compounds were available, as was DDT, in pure crystalline form. The activity of two was good and of the other three, fair. These compounds, their synonyms and relative potencies against *Drosophila* are listed on p. 302.

Methods

Each compound was tested against five insect species: the grain weevil, *Calandra granaria;* the flour beetle, *Tribolium confusum;* the flour moth, *Ephestia kuhniella;* the large milkweed bug, *Oncopeltus fasciatus;* and the German cockroach, *Blatella germanica.*

The grain weevils and flour beetles were cultured in wheat and flour respectively. Each culture was cleared of all adults every 10 days so that these insects, used for testing, were from 0 to 10 days old from emergence. The flour moth was cultured on untreated peanuts and all adults removed every week

A. DDT: 1,1-*bis*(4-chlorophenyl)-2,2,2-tri-
chloroethane

B. FLUORO-DDT: 1,1-*bis*(4-fluorophenyl)-
2,2,2-trichloroethane

(1)   Cl—⟨ ⟩   H—C—CCl₃

                     Cl—⟨ ⟩

(1/5 – 2/5)   F—⟨ ⟩   H—C—CCl₃

                     F—⟨ ⟩
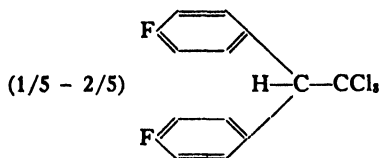
C. ACETOXY-CARBINOL-DDT: 1-(4-chloro-
phenyl)-2,2,2-trichloroethyl acetate

D. CARBINOL-DDT: 1-(4-chlorophenyl)-
2,2,2-trichloroethanol

                              O
                              ‖
                         O—C—CH₃
(1/10)   Cl—⟨ ⟩—C—CCl₃
                         |
                         H

                              OH
                              |
(1/50)   Cl—⟨ ⟩—C—CCl₃
                              |
                              H
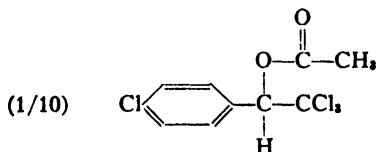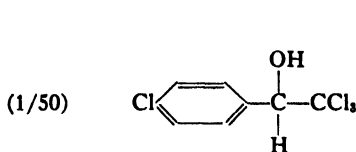
E. METHYL-DDT: 1,1-*bis*(4-methylphenyl)-
2,2,2-trichloroethane

F. MONOCHLOR-DDT: 1-(4-chlorophenyl)-
1-phenyl-2,2,2-trichloroethane

(1/100)   CH₃—⟨ ⟩   H—C—CCl₃

           CH₃—⟨ ⟩

(1/100)   Cl—⟨ ⟩   H—C—CCl₃

           ⟨ ⟩
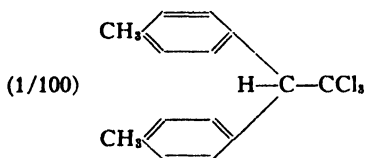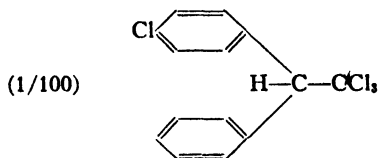
for testing.   The milkweed bugs were reared on milkweed seed and egg masses removed at biweekly intervals; these eggs were observed for hatching and the nymphs were used when from four to seven days old.   Cocoon-bearing cockroaches were cultured on fox chow; the nymphs were used 14 days after emergence from the cocoons.   No attempt was made to determine the exact instar of milkweed bugs or cockroaches.

For all species except *Blatella germanica* and for all compounds including DDT itself, test surfaces were prepared by wetting the internal surfaces of half-pint milk bottles with acetone solutions at appropriate strengths.   The bottles were allowed to dry at room temperature for four to five hours.   From 10 to 40 insects were placed in each bottle.   The bottles were stoppered with cotton wool plugs moistened in 5% aqueous molasses solution and incubated at 30° C.   The number of insects per bottle, the number of bottles, and the period of incubation varied with the species.   All compounds were tested against a single species at the same time.   Mortality counts were made at the end of the incubation period.   The testing method was essentially that used in earlier work (2) for *Drosophila.*

For the cockroach a different method was used.   Approximately 50 mgm. of DDT or compound (as a finely divided powder) was placed at the bottom of half-pint milk bottles and the requisite number of roaches added.   The bottles were stoppered and incubated as for the other species.   The incubation period was 48 hr.

Results

Preliminary tests showed that the preparation of test bottles with a solution of DDT containing 2.5 mgm. per 100 cc. of acetone would give a percentage mortality above 30 and below 80 for all insects except *Blatella germanica*, providing that the time of incubation was adjusted accordingly. This concentration was taken as the standard for comparison. *Calandra granaria* and *Tribolium confusum* required five and seven days' incubation respectively and *Oncopeltus fasciatus* and *Ephestia kuhniella* 40 hr. The related compounds were used at strengths equivalent in insecticidal potency to that of the standard as based on results with *Drosophila* i.e., fluoro-DDT, 2.5 × 5 mgm. %; acetoxy-carbinol-DDT, 2.5 × 10 mgm. %; carbinol-DDT, 2.5 × 50 mgm. %; and methyl-DDT and monochlor-DDT, 2.5 × 100 mgm. %. At such a concentration the carbinol produced a 100% mortality against *Oncopeltus fasciatus*. The test was repeated for this insect using the carbinol at 50 mgm.%, 5 mgm. %, and 2.5 mgm. %. The test was also repeated for the fluorine analogue using the original concentration.

The same tests with *Blatella germanica* showed that this roach was not killed in a reasonable time even when DDT at a concentration of 50 mgm. % was used to prepare test bottles. Consequently this insect was exposed to pure DDT and pure compounds as already described.

The larvae of *Ephestia kuhniella* were tested in the same manner as the adults. There was no mortality and all the larvae pupated and emerged as adult moths.

Table I summarizes the results, giving average percentage mortalities for each compound against each species (except the larval *Ephestia kuhniella*). Control mortalities, in untreated bottles, were 20% for *Calandra granaria*, 6% for *Tribolium confusum*, and 0% for the other three species. Table II expresses the potencies relative to DDT of each compound to each species.

With each compound there were examples of anomalous action. The fluorine analogue was twice as effective as DDT against *Blatella*. The acetoxy-carbinol, inactive against *Oncopeltus*, *Ephestia*, and *Tribolium*, was relatively potent against *Blatella*. The carbinol was equal in potency with DDT against *Oncopeltus*. Methyl-DDT and monochlor-DDT, maintaining their moderate potency in general, were inactive against *Tribolium*.

Discussion

The findings with fluoro-DDT correspond with those of Domenjoz (6) and Müller (10). Not only did they find that it maintained its high activity against all species tested but that it was more effective than DDT against *Calliphora*, *Calandra*, and *Formica*. Its specificity against the roach, as found in the present work, is interesting for the classical roach poison, sodium fluoride, has been shown to act as a contact rather than as a stomach insecticide (7). However, Busvine (3) found fluoro-DDT much less effective than DDT against *Pediculus* and *Cimex* and the results of Proverbs and Morrison (13) suggest that it decomposes or volatilizes readily.

TABLE I

PERCENTAGE MORTALITIES AMONG FIVE INSECT SPECIES ON EXPOSURE TO VARIOUS
CONCENTRATIONS OF DDT AND RELATED COMPOUNDS

Compound	Conc. in mgm. %	Insect species and percentage mortalities with no. of insects per test and no. of tests				
		Calandra granaria 40 × 10	*Tribolium confusum* 40 × 5	*Ephestia kuhniella* 10 × 5	*Oncopeltus fasciatus* 40 × 8	*Blatella germanica** 10 × 3
DDT	2 5 2.5	78	29	62	51 58	37
Fluoro-DDT	12 5 12.5	63	24	66	55 49	80
Acetoxy-carbinol-DDT	25.0	38	0	0	0	30
Carbinol-DDT	125 0 50.0 5.0 2.5	27	17	46	100 100 76 52	0
Methyl-DDT	250.0	52	0	68	42	17
Monochlor-DDT	250 0	73	0	60	31	7

* Tested at the same concentration of all compounds.

TABLE II

POTENCIES OF FIVE COMPOUNDS AGAINST DIFFERENT INSECT SPECIES RELATIVE TO DDT

Species	Fluoro-DDT	Acetoxy-carbinol-DDT	Carbinol-DDT	Methyl-DDT	Monochlor-DDT
*Drosophila**	Good	Good	Fair	Fair	Fair
Oncopeltus	Good	Nil	Good	Fair	Fair
Ephestia	Good	Nil	Fair	Fair	Fair
Calandra	Good	Fair	Poor	Fair	Fair
Tribolium	Good	Nil	Poor	Nil	Nil
Blatella	Very good	Good	Nil	Fair	Poor

* See (2). Very good—relative potency more than 1; good - relative potency 1 to 1/10th; fair—relative potency 1/50th to 1/100th; poor - relative potency less than 1/100th; nil—no activity.

Carbinol-DDT was more effective than DDT against *Calliphora*, *Formica*, and *Pediculus* according to Domenjoz (6) but Busvine (3) does not agree with the findings towards the last-named species. Methyl-DDT has been found as potent as DDT against *Culex* (12), *Carpocapsa* (16), and *Calliphora* (10); so has monochlor-DDT against *Calliphora* (10). The carbinol acetate has not been examined by these authors.

Examples of inactivity of other compounds towards certain species are conspicuous throughout the work of Domenjoz (6) and of Müller (10). Haller (8) mentions that methyl-DDT and methoxy-DDT (1,1-*bis*(4-methoxyphenyl)-2,2,2-trichloroethane) have specificity against the codling moth and lack of it against the corn borer while DDD (1,1-*bis*(4-chlorophenyl)-2,2-dichloroethane) showed the reverse selectivity. Turner (18) found methoxy-DDT more effective than DDT against the Mexican bean beetle at one-eighth the concentration. Busvine (3, 4) found methoxy-DDT as effective against *Cimex* as DDT though only one-third as toxic to *Pediculus*.

The reasons for these specificities are likely to be found in the nature of the insect integument and the chemophysical properties of the insecticidal compound. The outermost layer of the integument consists of a mixture of fats and waxes and may be further covered with fatty films of secretion as it is in the cockroach (20). It has been shown that pyrethrins enter the integument faster if this lipoid is first removed (21). The inner layer varies in thickness and degree of impregnation with protein, chitin, fats, waxes, and phenols (5, 20), not only from place to place in the individual but even between closely related species (20). The importance of such variation is suggested by the fact that both the gamma isomer of hexachlorocyclohexane and DDT are much more toxic to newly emerged *Calliphora* than to the older individuals (15, 17); it is following on emergence that the integument undergoes chemical changes towards impermeability (20). The only pertinent information available on the present compounds is that they are all as or more oil soluble than DDT, the fluorine analogue especially so (6, 10, 11). A histochemical investigation of the integument of species differing in susceptibility to DDT and related compounds while subject to the contact action of these substances should be instructive.

The order of increasing susceptibility towards DDT was *Blatella, Tribolium, Calandra, Ephestia, Oncopeltus*, and *Drosophila*. The dipteran *Calliphora* was the most sensitive insect to the action of 20 compounds tested by Domenjoz (6) and to that of 37 tested by Müller (10). Certain compounds were inactive against one or more species in the present work although active against *Drosophila*. It appears that the use of this insect as a detector or indicator of insecticidal activity is a valid procedure.

Acknowledgment

References

BARRY, G. T. Synthesis of DDT homologs. In preparation.

BROWNING, H. C., FRASER, F. C., SHAPIRO, S. K., GLICKMAN, I., and DUBRÛLE, M. The biological activity of DDT and related compounds. Can. J. Research, D, 26 :282-300. 1948.

3. BUSVINE, J. R. Insecticidal tests with analogues of DDT. J. Soc. Chem. Ind. 65 : 356-360. 1946.

BUSVINE, J. R.   Insecticidal action of DDT.   Nature, 156 : 169-170.   1945.

5. DENNEL, R.   A study of insect cuticle: the formation of the puparium of *Sarcophaga falculata* Pand. (Diptera).   Proc. Roy. Soc. London, B, 134 : 79-110.   1947.

6. DOMENJOZ, R.   Action biologique de quelques dérivés du DDT.   Helv. Chim. Acta, 29 : 1317-1322.   1946.

7. GRIFFITHS, J. T. and TAUBER, O. E.   Evaluation of sodium fluoride as a stomach poison and as a contact insecticide against the roach, *Periplaneta americana* L.   J. Econ. Entomol. 36 : 536-540.   1943.

8. HALLER, H. L.   Wartime development of insecticides.   Ind. Eng. Chem. 39 : 467-473.   1947.

9. KIRKWOOD, S. and DACEY, J. R.   Synthesis of some analogs of 1,1-*bis*-(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT).   I. Three fluorine analogs.   Can. J. Research, B, 24 : 69-72.   1946.

10. MÜLLER, P.   Über Zusammenhänge zwischen Konstitution und insektizider Wirkung I.   Helv. Chim. Acta, 29 : 1560-1580.   1946.

11. OETTINGEN, W. F. VON and SHARPLESS, N. E.   The toxicity and toxic manifestations of 2,2-*bis*-(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) as influenced by chemical changes in the molecule.   J. Pharmacol. 88 : 400-413.   1946.

12. PRILL, E. A., SYNERHOLM, M. E., and HARTZELL, A.   Some compounds related to the insecticide "DDT" and their effectiveness against mosquito larvae and houseflies.   Contr. Boyce Thompson Inst. 14 : 341-353.   1946.

13. PROVERBS, M. D. and MORRISON, F. O.   The relative insecticidal activities of DDT and related organic molecules.   Can. J. Research, D, 25 : 12-44.   1947.

14. ROSS, J. H., BOYER, R., GARMAISE, D. L., BARRY, G. T., and WHITE, H. L.   Progress reports to the National Research Council, Canada.   Unpublished.   1944-46.

15. SAVIT, J., KOLLROS, J. J., and TOBIAS, J. M.   Measured dose of gamma hexachlorocyclo-hexane (y666) required to kill flies and cockroaches and a comparison with DDT.   Proc. Soc. Exptl. Biol. Med. 62 : 44-48.   1946.

16. SIGLER, E. H. and GERTLER, S. I.   Toxicity of diaryl trichloroethanes and dichloroethyl-enes to codling moth.   J. Econ. Entomol. 37 : 845.   1944.

17. TOBIAS, J. M., KOLLROS, J. J., and SAVIT, J.   Relation of absorbability to the com-parative toxicity of DDT for insects and mammals.   J. Pharmacol. 86 : 287-293.   1946.

18. TURNER, N.   Organic insecticides for control of the Mexican bean beetle.   Connecticut Agr. Expt. Sta. Bull. 511.   In press.   1947.

19. WHITE, H. L.   Synthesis of DDT homologs.   In preparation.

20. WIGGLESWORTH, V. B.   The principles of insect physiology.   E. P. Dutton & Co., Inc., New York.   1939.

21. WIGGLESWORTH, V. B.   Some notes on the integument of insects in relation to the entry of contact insecticides.   Bull. Entomol. Research, 33 : 205-218.   1942.

ASCARIS LUMBRICOIDES INFECTION IN GUINEA PIGS WITH SPECIAL REFERENCE TO EOSINOPHILIA AND RESISTANCE[1]

By A. Murray Fallis[2]

Abstract

Clinical symptoms of *Ascaris* infection were produced in guinea pigs by feeding several thousand eggs. Such infections caused a temporary loss in weight and severe congestion of the lungs but no elevation in temperature was observed. An eosinophilia was associated with infection and it reached higher levels following repeated infections. Injections of antigen caused a temporary rise in the number of eosinophiles. Guinea pigs developed a resistance as a result of infection. Some resistance was retained for at least 15 weeks following infection. A slight passive resistance resulted from injections of large quantities of serum from resistant animals and from injections of a liver extract prepared from resistant animals. The resistance was apparent from the amount of congestion in the lungs and the number and size of the larvae recovered from the lungs. The eosinophiles per se were not responsible for the resistance observed. It appeared that the body defences, in resistant animals, acted against the parasites before they reached the liver and more especially before they reached the lungs.

Work, of several investigators, has shown that animals that have been infected with *Ascaris* spp. are resistant to subsequent infection with the same species. Opinions differ regarding the duration and nature of the resistance and quantitative measurements have not always been made.

Hadwen (6) postulated, from his study of ascariasis in horses, that "immunity to ascarids is stimulated and increased by repeated attacks of these parasites". He suggested that, in addition to other antisubstances produced in the body to neutralize the cast off products of the worms, some substance is secreted by the eosinophiles that is detrimental to the worms. Morgan (9), in his work on *Ascaris* infection in hogs felt the evidence for an acquired immunity was far from conclusive. Wagner (15) showed, from experimental studies on mice infected with *Ascaris lumbricoides*, that fewer larvae were found in the livers and lungs of animals that had been infected previously. De Boer (4) found that hogs that had been infected previously were resistant to subsequent infection. Roberts (12) found that hogs that were exposed to continuous infection developed an immunity that he thought was concerned with the leucocytes, chiefly the eosinophiles, and the reticuloendothelial system. He thought the body defences were mobilized at the intestinal wall, liver, and lungs, especially at the intestine.

An extensive investigation of the cellular response in guinea pigs that had acquired resistance to infection with pig *Ascaris* was carried out by Kerr (8). He observed that a larger number of guinea pigs survived lethal doses of *Ascaris*

eggs if they had received sublethal doses one week previously. He thought the resistance might be of short duration, lasting for only a short time after the larvae had disappeared from the body of the host. There was some indication also that the more sublethal infections a guinea pig received the more solid was the immunity. He noticed that the larvae did not grow as fast in the livers of resistant guinea pigs and there was less hemorrhage in the lungs because he thought fewer larvae had reached the lungs, and, moreover, they were being destroyed there.

The present investigation was begun in order to study (1) the effect of the parasite on the host, (2) the eosinophilia associated with infection, (3) the resistance that is produced by infection.

Materials and Methods

Guinea pigs of comparable sizes were selected for each experiment. *Ascaris* eggs, obtained by dissection from the distal portion of the uterus of mature female worms, were incubated in 0.5% formalin at 30° C. for at least three weeks. It was assumed that eggs were infective after this incubation.

The number of eggs fed each animal was determined by a dilution method or by counting the total number (when the dose contained fewer than one hundred eggs). The eggs were collected on small disks of fine filter paper, which were then placed in No. 5 gelatin capsules and fed to the guinea pigs. The capsules were swallowed more readily if softened with water and coated with sugar.

Blood smears and differential counts of 200 white blood cells were made in the usual manner.

Larvae were isolated from the tissues by digesting the latter in pepsin and hydrochloric acid at 37° C. Digestion of liver tissue was improved by comminution in the Waring Blendor (Fallis (5)) before adding the enzyme. Larvae were sometimes killed by digestion as Ransom and Cram (11) have pointed out. However, there was no evidence to show that they were digested to such an extent that they could not be recognized. Moreover the error should be similar in each experiment. The number of larvae remaining in the residue was counted in a Petri dish that had been ruled in squares. Larvae were measured following fixation in hot alcohol.

Ascaris antigen was prepared by drying whole specimens in a vacuum oven at 37° C., grinding them in a mortar, and suspending the product in phenolized saline. This antigen was stored in the refrigerator. Injections of antigen were given by the intraperitoneal route.

An extract was prepared as follows from the livers of a number of guinea pigs that were sacrificed 11 days after they were fed several thousand *Ascaris* eggs. The livers were removed and comminuted in a Waring Blendor with an equal volume of saline for two minutes. The resulting emulsion was

stirred continuously and fast frozen in a mixture of alcohol and solid carbon dioxide. It was then removed and thawed. The freezing and thawing were carried out three times. The mixture was then centrifuged and the supernatant was filtered through a Büchner filter. A volume of absolute alcohol was added to the filtrate to make the alcoholic concentration 80%. The addition of the alcohol produced a heavy precipitate that was allowed to settle for 16 hr. at room temperature. The precipitate was then thrown down by centrifuging and the supernatant was placed in a vacuum desiccator at 30° C. The supernatant was evaporated from 60 cc. to 20 cc. in 48 hr. A specific gravity determination of the remaining liquid indicated that the alcohol had been removed. Water was added to make the volume up to 30 cc. and the liquid was filtered through a Berkefeld candle. The filtrate was stored in the refrigerator. A liver extract was prepared at the same time, and in a similar way, from a group of guinea pigs that had not been infected.

Serum was collected from the blood of normal guinea pigs and from guinea pigs that had been infected. The so-called 'normal' serum was obtained from the blood of a group of noninfected guinea pigs. The 'resistant' serum was obtained from the blood of guinea pigs that had been infected three weeks previously by feeding each of them 20,000 to 30,000 *Ascaris* eggs. These sera were filtered through Berkefeld candles and stored in the refrigerator.

Rate of Growth, Temperature, and Eosinophile Response in Guinea Pigs Fed *Ascaris* Eggs

The rate of growth, temperature, and eosinophile level was followed in guinea pigs that received the following treatment: 10 *Ascaris* eggs daily for 14 days; 20 eggs daily for 14 days; single dose of several thousand eggs; no infection. There were five guinea pigs in each group.

No significant elevation in temperature was observed in any of the guinea pigs during the infection and for one week following the last dose of eggs. This observation differs from that of Roberts (12) who found an elevation in the temperature of hogs fed large doses of eggs over a prolonged period. Guinea pigs that received 10 or 20 eggs daily made similar gains in weight to those in the control group, which received no eggs. The percentage of eosinophiles in the blood was also similar with the exception of that in a single animal fed 20 eggs daily in which the eosinophiles increased to about 15% following infection. The guinea pigs that received a single dose of several thousand eggs began to lose weight four to five days following the ingestion of the eggs (Fig. 1) and at the same time there was an increase in the eosinophiles in the peripheral blood. Kerr (8) and Roberts (12) have shown also that an eosinophilia is associated with infection in guinea pigs. Those pigs that survived the infection began to gain weight about 10 days after they had been fed the *Ascaris* eggs (Fig. 1). There was a marked decline in the number of eosinophiles about 12 days after the ingestion of the eggs.

The lungs of one guinea pig that had been fed several thousand eggs, and that died eight days after infection, showed extensive congestion and a large number of eosinophiles (Fig. 2).
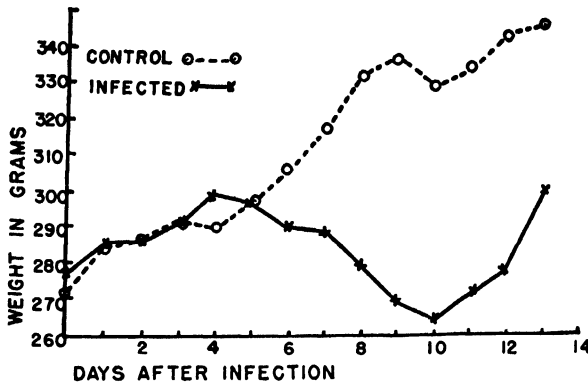
FIG. 1. *Average rate of growth of four guinea pigs following infection by feeding several thousand eggs compared to that of five guinea pigs that received no eggs.*

Rate of Migration of the Larvae Through the Body

Sixteen guinea pigs weighing approximately 300 gm. were each fed about 13,000 eggs. Two of the guinea pigs were sacrificed daily, beginning three days after infection, to determine the rate of migration of the larvae to the lungs.

The maximum number of larvae was recovered from the lungs of the guinea pigs eight days after they had ingested the eggs. Relatively few of the larvae reached the lungs before the fifth day following infection. The total number of larvae migrating to the lungs was small in comparison with the number of eggs fed.

Resistance in Guinea Pigs Infected Previously

Thirty guinea pigs were infected with *Ascaris* by feeding each pig over 9000 eggs. The guinea pigs showed a decline in weight, following infection, similar to that illustrated in Fig. 1. There was also a marked rise in the number of eosinophiles in the blood following infection. The number returned to the normal level two to three weeks later. One month after this infection each guinea pig was dosed again with approximately 27,000 eggs. At the same time each of a second group of 30 guinea pigs was infected for the first time by feeding a similar number of *Ascaris* eggs to that given to the animals in the first group. Five other guinea pigs were kept as controls and received no *Ascaris* eggs.

The average percentage of eosinophiles in the peripheral blood of the guinea pigs in these three groups on successive days following infection is shown in Fig. 3. The average is based on counts from more animals at the beginning of the experiment than at the end as three or four of the pigs in each group were sacrificed daily commencing five days after their final infection.

PLATE I
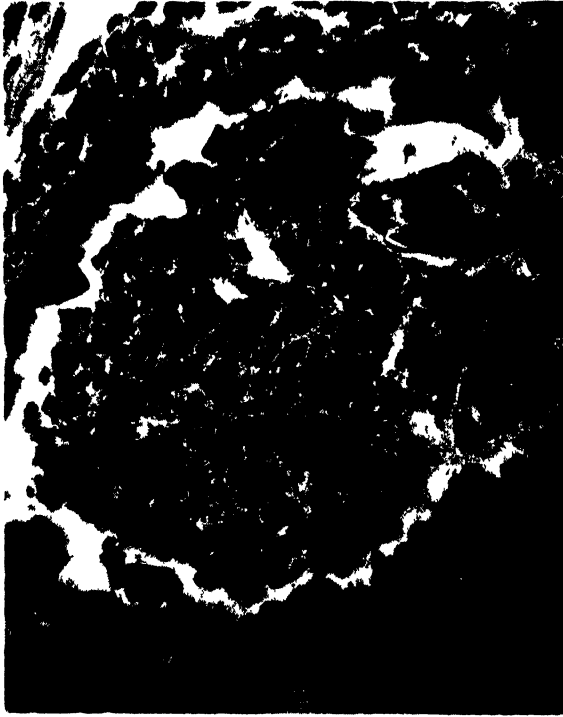
FIG. 2   Extensive eosinophilia in section of lung of guinea pig eight days after infection with Ascaris lumbricoides.

The gross appearance of the lungs of the guinea pigs in each group, with the exception of those from animals killed 5 and 10 days after infection, is illus-
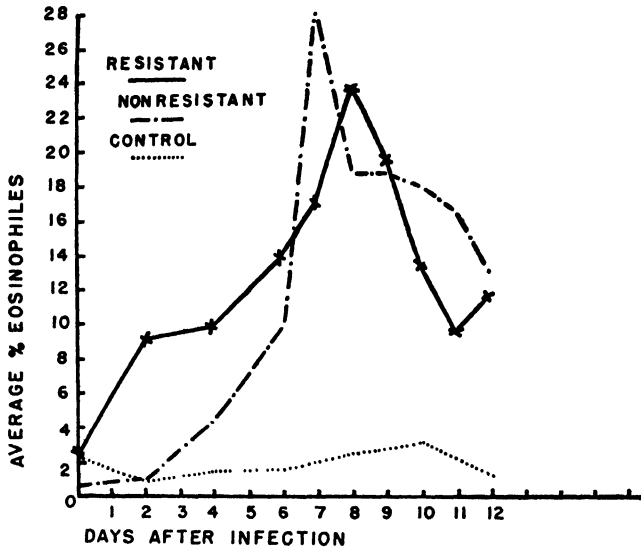
FIG. 3. *Average percentage of eosinophiles in the blood of resistant and nonresistant guinea pigs following infection.*

trated in kodachrome pictures (Fig. 4). The weight of the lung of each guinea pig was recorded also for comparison with the weight of the animal (Fig. 8).

The average number of larvae recovered from the lungs of the two groups of pigs on successive days following infection is illustrated in Fig. 9. It is apparent that fewer larvae reached the lungs of the pigs that had been infected only once, that is to say, guinea pigs infected with *Ascaris* are resistant to a second infection given one month after the first. In no instance, however, did the number of larvae recovered from the lungs nearly equal the number of eggs that had been fed. No explanation can be given for the recovery of fewer larvae from the guinea pigs in the nonresistant group on the seventh day than on either the sixth or eighth day after infection, but it will be observed (Fig. 4) that there was less congestion in the lungs of the guinea pigs killed on the seventh day.

The congestion in the lungs of the guinea pigs that had been infected for the first time was, in general, more marked than in those that had been infected more than once (Figs. 4 and 8) and was no doubt related to the number of larvae present (Fig. 9). It will be observed, from a comparison of Figs. 4 and 8, that the weights of the lungs of the nonresistant guinea pigs increased, in relation to their body weights, as the congestion in the lungs increased, and the weights of the lungs decreased as the congestion disappeared. The lungs from the resistant animals on the other hand were much lighter, in relation

TABLE I

AVERAGE SIZE *Ascaris* LARVAE IN LUNGS OF RESISTANT AND NONRESISTANT GUINEA PIGS ON DIFFERENT DAYS FOLLOWING INFECTION. THE FIGURES IN PARENTHESES INDICATE THE NUMBER OF MEASUREMENTS USED TO CALCULATE THE AVERAGES

Days after infection	Average size of larvae in mm.	
	Resistant	Nonresistant
5	0.4 (2)	0.53 (20)
6	0.55 (39)	0.88 (97)
7	0.89 (47)	1.1 (113)
8	1.1 (28)	1.3 (94)
9	1.2 (28)	1.4 (77)
10	1.3 (15)	1.5 (71)
11	1.3 (14)	1.4 (59)
12	1.5 (6)	1.5 (20)

to the weights of the animals, except on the fifth day after infection, which would seem to be related also to the extent of the congestion. It will be observed, however, that the lungs from some of the resistant animals, e.g. those sacrificed eight and nine days after infection, appeared larger than those from nonresistant animals sacrificed at the same time. This appearance is due to emphysema in the lungs of the resistant guinea pigs. A similar condition was not very apparent in the lungs of the nonresistant animals until later (10 to 12 days after infection) as will be seen from an examination of the photographs of the lungs Nos. 109 to 115 in Fig. 4. A close examination of the photograph of the lung from guinea pig No. 113 will reveal a finger impression on the left posterior lobe that was made purposely to illustrate the emphysematous condition. It was found also that the larvae were larger, on the average, in the lungs of the nonresistant animals than in the lungs of the resistant animals on any one day, except the 12th, following infection (Table I). This will be discussed later following the outline of another similar experiment.

FIG. 4.   *Gross appearance of lungs from resistant and nonresistant guinea pigs 6 to 12 days after infection.   Nos. 69 to 95—lungs from resistant guinea pigs.   Nos. 109 to 135—lungs from nonresistant guinea pigs.   No picture available of Nos. 75, 76, 78, 79, and 116 to 119.*

Appearance 6 days after infection.   Severe congestion in lungs of Nos. 132 to 135, some congestion in lungs of Nos. 92 to 95.

Appearance 7 days after infection.   Some congestion in lungs of Nos. 128 to 131, very little congestion in lungs of Nos. 88 to 91.

Appearance 8 days after infection.   Severe congestion in lungs of Nos. 124 to 127, no congestion but some emphysema in lungs of Nos. 84 to 87.

Appearance 9 days after infection.   Congestion in lungs of Nos. 120 to 123 less severe than previously and some emphysema.   Emphysema marked in lungs of Nos. 80 to 83.

Appearance 11 days after infection.   Marked emphysema in lungs of Nos. 109 to 112, emphysema slight in lungs of Nos. 70 and 73, lungs of 74 and 77 have almost normal appearance.

Appearance 12 days after infection.   Marked emphysema in lungs of Nos. 113 to 115, slight emphysema in lungs of Nos. 69 to 72.

PLATE II

FIG 4

PLATE III
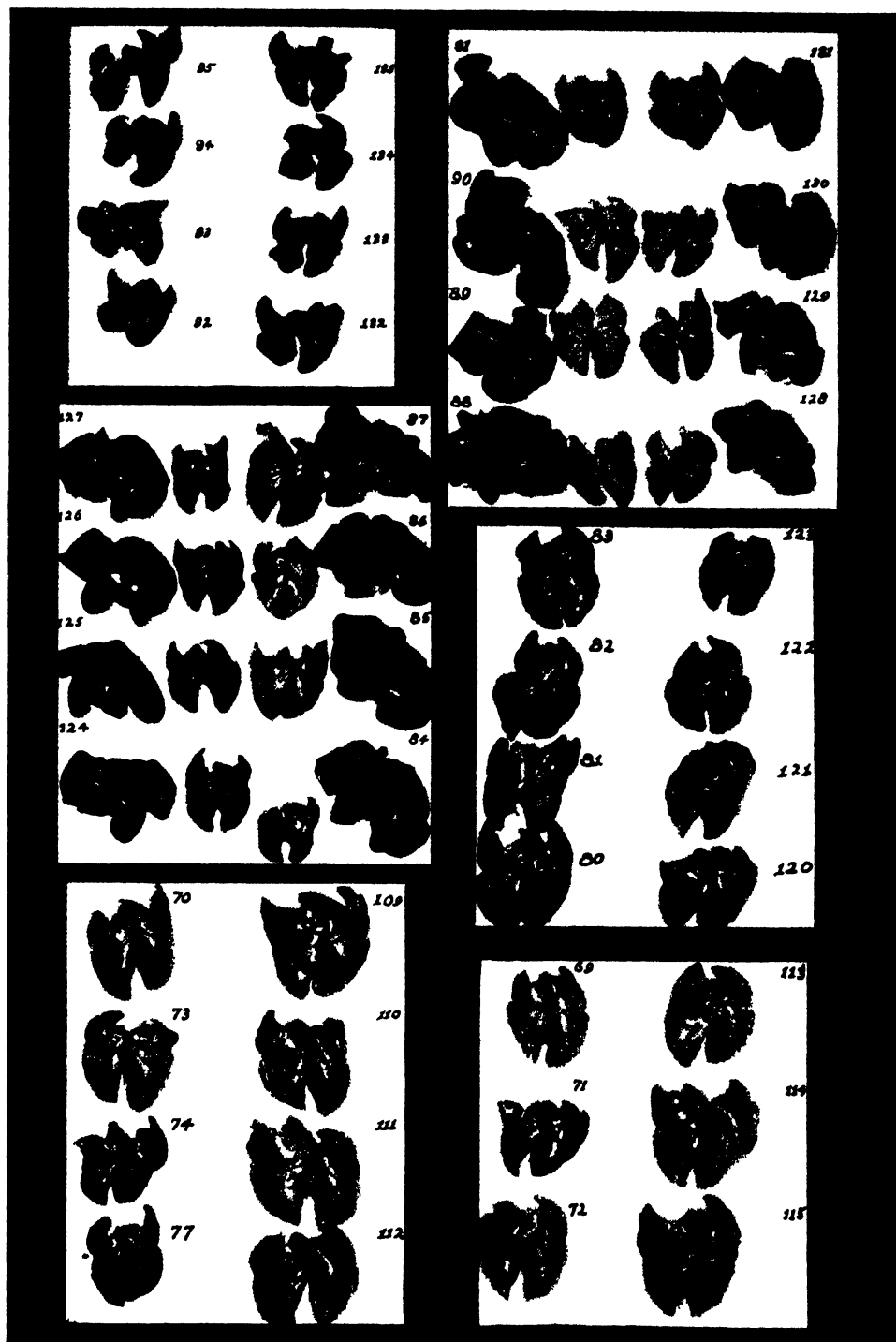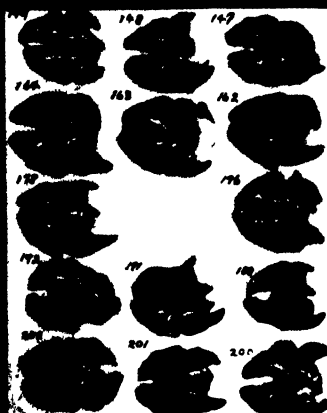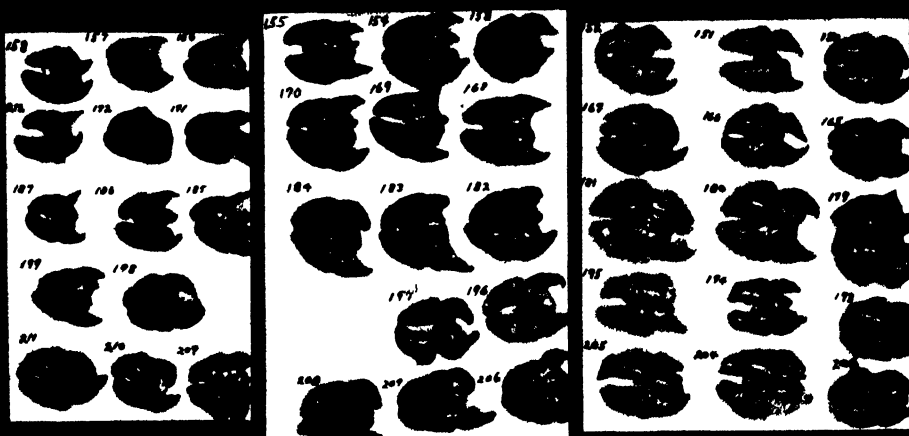
Fig 5
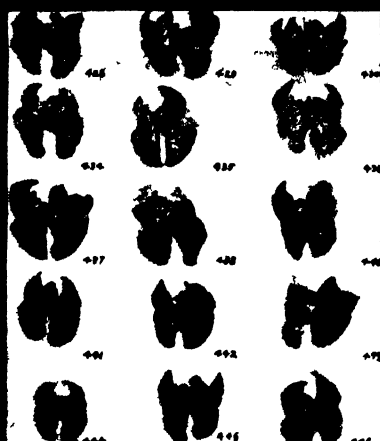
Fig. 6

Fig 7
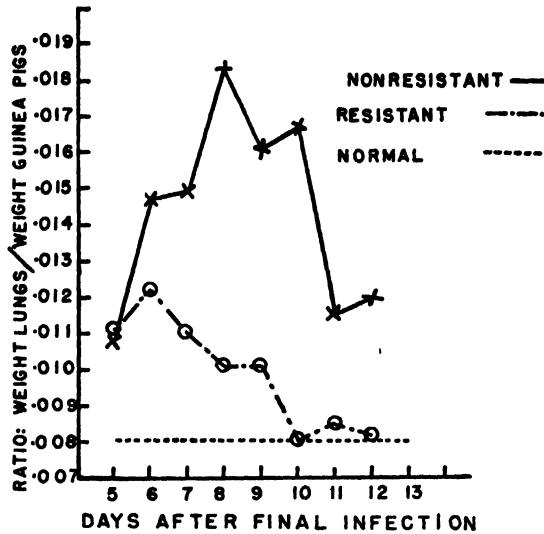
FIG. 8. *The relationship between the weights of the lungs in resistant and nonresistant guinea pigs and the weights of the animals during the course of infection with Ascaris. The weights on different days following infection are the averages for the three or four animals that were sacrificed on successive days following infection.*

It is obvious from these observations that guinea pigs that have been infected previously with *Ascaris lumbricoides* are somewhat resistant to subsequent infections. It will be observed also (Fig. 3) that the average percentage of eosinophiles in the guinea pigs in the resistant group increased more rapidly than in those that had been infected for the first time.

FIG. 5. *The lungs of guinea pigs that had repeated infections, a single infection, and injections of antigen.*

Appearance 6 days after infection. Nos. 156 to 158 (IA) and 171, 172, 212 (IB), and 185 to 187 (II) show marked congestion although less in IA. Lungs of guinea pigs Nos. 198 to 199 (IIIA) and Nos. 209 to 211 (IIIB) appear almost normal.

Appearance 7 days after infection. Nos. 153 to 155 (IA), 168 to 170 (IB), and 182 to 184 (II) show marked congestion although slightly less in IA. Nos. 196 to 197 (IIIA) and 206 to 208 (IIIB) appear almost normal.

Appearance 8 days after infection. Nos. 150 to 152 (IA), Nos. 165 to 167 (IB), and 179 to 181 (II) show some congestion and emphysema especially in II. Nos. 193 to 195 (IIIA) and 203 to 205 (IIIB) show some emphysema.

Appearance 9 days after infection. Nos. 147 to 149 (IA), 162 to 164 (IB), and 176 and 178 (II) show slight congestion and some emphysema. Nos. 188, 191, 192 (IIIA), and 200 to 202 (IIIB) appear normal.

Appearance 10 days after infection. Nos. 144 to 146 (IA), 159 to 161 (IB), and 173 to 175 (II) show emphysema and slight congestion.

FIG. 6. *Gross appearance of lungs, seven days after infection, from guinea pigs that were (a) resistant as a result of previous infection (Nos. 428 to 430, 434 to 436); (b) infected for the first time (Nos. 437, 438, 440); (c) receiving injections of liver extract prepared from normal guinea pigs (Nos. 441 to 443); (d) receiving injection of liver extract prepared from resistant guinea pigs (Nos. 444 to 446).*

FIG. 7. *Appearance of lungs, seven days after infection, from guinea pigs that had (a) received injections of 'normal' serum at the time of infection (Nos. 542 to 550); (b) received injections of 'resistant' serum at the time of infection (Nos. 551 to 559); (c) received no injections (Nos. 560 to 568).*
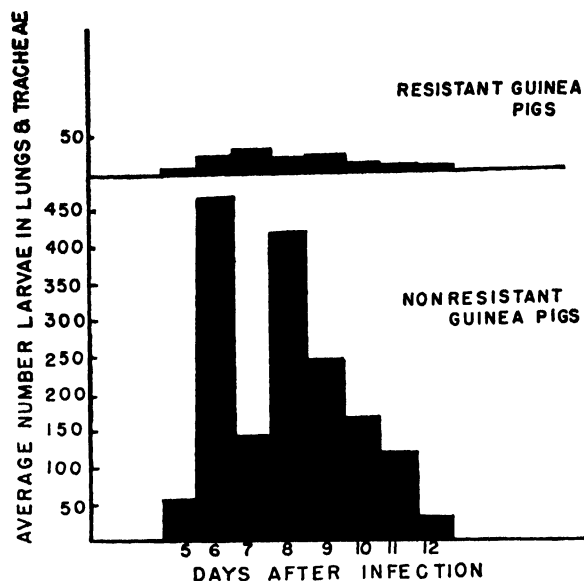
FIG. 9. *Average number of Ascaris larvae recovered from the lungs and tracheae of four resistant and four nonresistant guinea pigs on successive days following infection, except on the 12th day on which only three animals in each group were examined.*

Resistance in Guinea Pigs Following Repeated Infections and in Those Showing a High Eosinophilia Resulting from Injection of Ascaris Antigen

Three groups of guinea pigs were selected so that there were 30, 30, and 24 animals respectively, in each group. The animals in Group I received injections of *Ascaris* antigen as shown in Fig. 10, those in Group II were kept as controls; each of those in Group III was fed 8000, 15,000 and 15,000 *Ascaris*
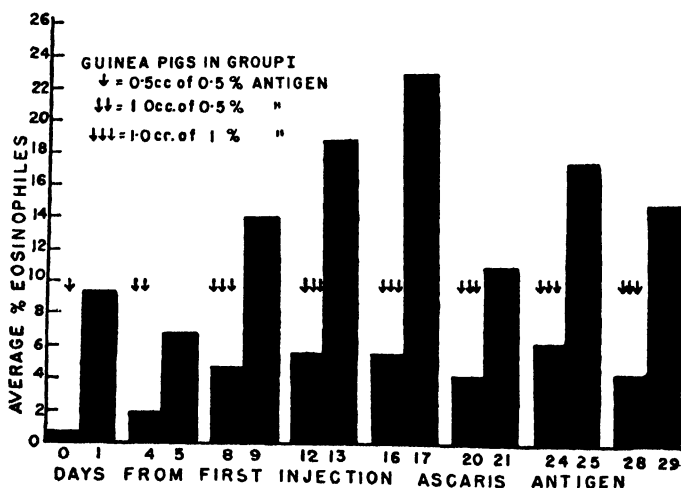
FIG. 10. *The average eosinophilia in 10 guinea pigs before and after repeated intraperitoneal injections of phenolized Ascaris antigen.*

eggs at intervals of 11 days respectively, as indicated in Fig. 11. Forty days after the experiment was begun each of the guinea pigs was fed approximately 25,000 *Ascaris* eggs. Fifteen of the animals in Group I (IA) and 12 of the animals in Group III (IIIA) received six daily injections of 0.5 cc. antigen beginning at the time of this infection.
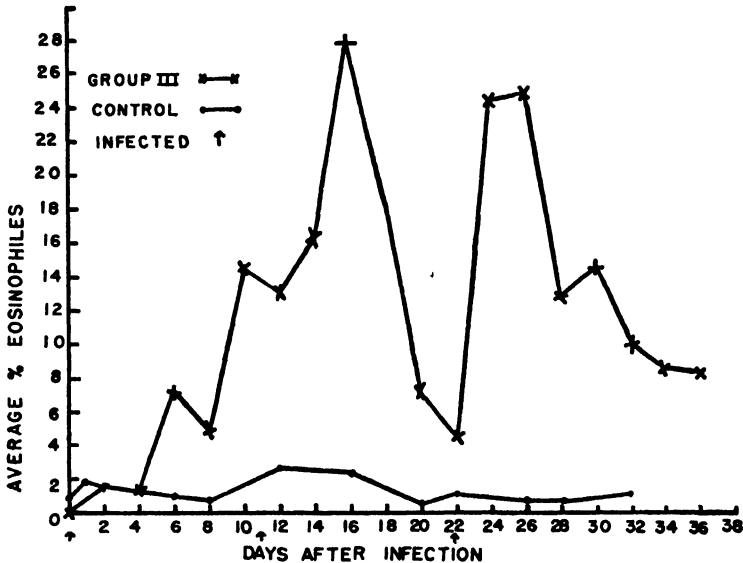
FIG. 11. *Average percentage of eosinophiles in 24 guinea pigs following repeated infections with Ascaris at intervals of 11 days, together with the average for five guinea pigs that were not infected.*

A high eosinophilia was produced by infections (Figs. 11 and 12). The injections of antigen also produced an eosinophilia (Fig. 10) but four days later it was considerably lower. However, a gradual rise in the eosinophile level was noticed following repeated injections for 16 days (Fig. 10). It will be observed that the average percentage of eosinophiles in those guinea pigs (Group IA) that received injections of antigen at the time of infection, as well as a previous series of injections, remained at a higher level than in the guinea pigs (Group IB) that did not receive injections at the time of infection (Fig. 12). The guinea pigs (Group IIIA) that had been repeatedly infected and that had received also injections at the time of their final infection, showed an initial rise in eosinophiles above that in the animals (Group IIIB) that did not receive injections, but subsequently the eosinophilia was higher, on the average, in the animals in the group IIIB.

The susceptibility of the guinea pigs to infection was compared by sacrificing three or four of the animals in each group on the 6th to 10th days inclusive following this final infection. The gross appearance of the lungs of the guinea pigs was recorded in kodachrome pictures (Fig. 5).

The largest number of larvae was recovered from the guinea pigs in Group II, i.e., the control group, which had been infected only once (Table II). Few

TABLE II

Days after infection	Group IA, antigen injections prior to and during infection	Group IB, antigen injections prior to infection	Group II, control	Group IIIA, infected previously and received antigen	Group IIIB, infected previously
6	13	23	21	1	1
7	32	35	69	1	1
8	43	138	393	—	1
9	33	105	93	1	1
10	137	61	218		

parasites were found in the animals in Group III, i.e., those that had received three infections, the first 40 days, and the last 18 days, before the final one. There was no significant difference in the number of parasites found in the animals in Group IIIA, which had received injections of antigen, compared with those in IIIB, which did not receive antigen. Fewer parasites were recovered, on the average, from the guinea pigs in Group IA than from those in Group IB. The former had received injections of antigen for six days following their infection as well as a series of eight injections at four day intervals at the beginning of the experiment. The animals in Group IB, on the other hand, had received only the series of injections at the beginning. A comparison of the gross appearance of the lungs from the guinea pigs in the different groups (Fig. 5) with the number of larvae recovered (Table II) illustrates the relationship between gross lesions and the number of larvae present.

The results confirm those of the previous experiment and show that guinea pigs that have been infected previously with *Ascaris lumbricoides* show a strong resistance to subsequent infection acquired 18 days later. There was also a slight indication that injections of *Ascaris* antigen, especially when administered before and during infection, may have produced some resistance in the guinea pigs receiving them. The animals that had received injections at the time of infection (IA) were more resistant than those that had not received these injections (IB). The resistance in the animals that had received injections of antigen, i.e. Group IA and IB, was not as complete as in those animals that had been immunized by reinfection, i.e., Group III. The high eosinophilia (Figs. 11, 12) in the resistant guinea pigs (Table II, Fig. 5) is apparent. It is evident also, from a comparison of the eosinophilia, in the animals in Groups IB and II and the number of larvae recovered from the two groups (Table II) that other factors are involved in resistance. The animals in Group IB showed some resistance and yet the eosinophile level was close to that observed in the animals (Group II) that were presumably nonresistant to *Ascaris* infection.

Duration of Resistance and Effects of Injections of *Ascaris* antigen

Eighty-four guinea pigs ranging in weight from 250 to 500 gm. were divided into four groups containing 24, 24, 24, and 12 pigs respectively. The guinea pigs in the different groups were infected with *Ascaris* eggs as indicated in
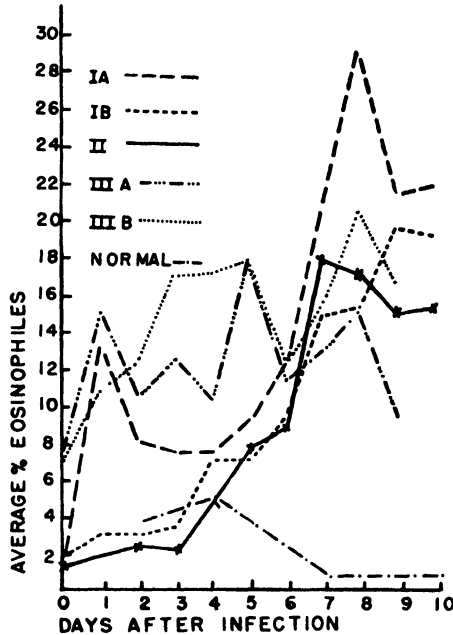
FIG. 12. *A comparison of the eosinophilia produced by infection with Ascaris in guinea pigs that had received the following treatment prior to this infection.*

Group IA — *injections of antigen prior to, and during infection;*

IB — *injections of antigen prior to infection;*

II — *control, no previous infection;*

IIIA — *previous infections, as well as injections of antigen at the time of final infection;*

IIIB — *previous infections.*

The averages are based on counts from five animals in each group except on the last day when there were only three animals left in each group.

Fig. 13. The animals in Group A were given three immunizing infections at intervals of nine and seven days respectively and four of the animals in this group received a fourth infection one week later. The animals in Group B were given a single infection and nine days later four of them were given a second infection comparable to that given to the four animals in Group A. The guinea pigs in Group C constituted the control group. Four of these animals were given a single infection at the same time as the four animals in Groups A and B received their final infections. Four of the animals in Group D were given a single infection as well as 1 cc. intraperitoneal injections of 1% antigen on the day prior to infection and for the following seven days. The four pigs in each group were sacrificed eight days after these infections.

Four additional guinea pigs in Groups A, B, and C were infected at intervals of 3, 5, 7, 10, and 15 weeks, respectively, following the final immunizing infections that had been given to the animals in Groups A and B.   Guinea
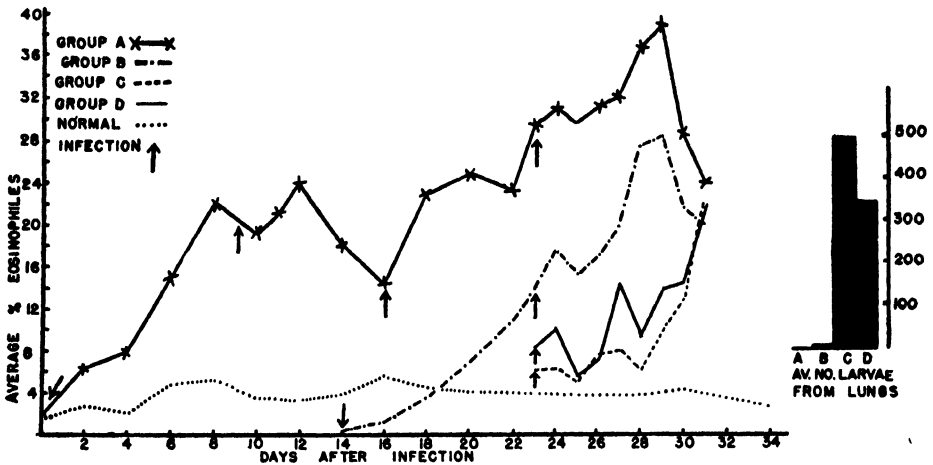
FIG. 13.   *Eosinophilia in guinea pigs in which infections were superimposed and a comparison with that observed in guinea pigs infected for the first time and in those receiving injections of antigen.   The average number of larvae recovered from the lungs of four of the animals in each group, eight days after their final infection, is shown also.*

pigs from Group D were infected at the 7 and 15 week intervals.   The animals in this group received intraperitoneal injections of antigen at four day intervals and daily during infection.   The four guinea pigs in each group were sacrificed eight days after infection.
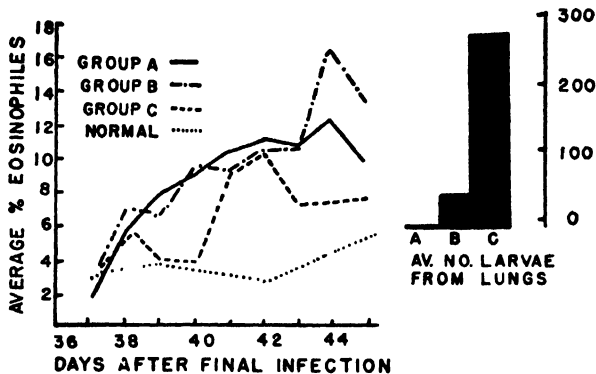
FIG. 14.   *Comparison of the eosinophilia produced by infection in nonresistant guinea pigs and in guinea pigs that were resistant as a result of infections given three weeks previously. The average number of larvae recovered from the lungs of four of the animals in each group, eight days after their final infection, is shown.*

An eosinophilia was associated with the infections (Figs. 13 to 18).   It was more pronounced when infections were superimposed (Groups A and B, Fig. 13).   This resembles the result obtained by Bachman and Rodriguez

Molina (1) with superinfections with *Trichinella spiralis*. It differs from the results of the work by Brown and Otto (3), with hookworms, in which they found that the addition of new worms did not necessarily induce an eosinophilia. In some instances (Fig. 16), the eosinophile level was high in the vaccinated guinea pigs, but in other cases it was similar to that in the unvaccinated animals (Fig. 18).
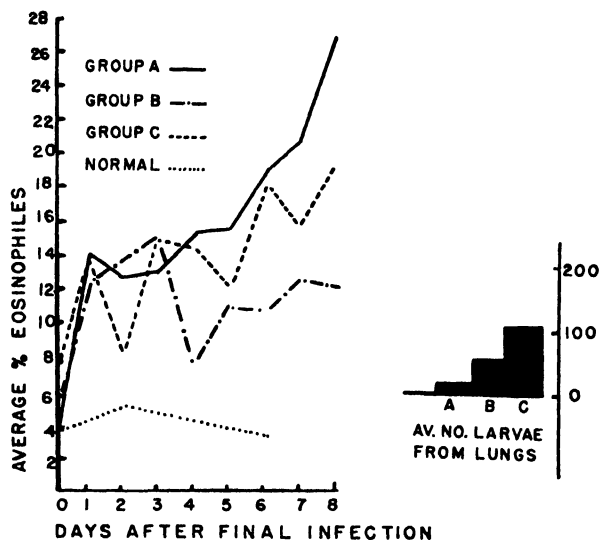
FIG. 15. *Similar to Fig. 13—except guinea pigs in resistant group had received [last immunizing infection five weeks previously.*
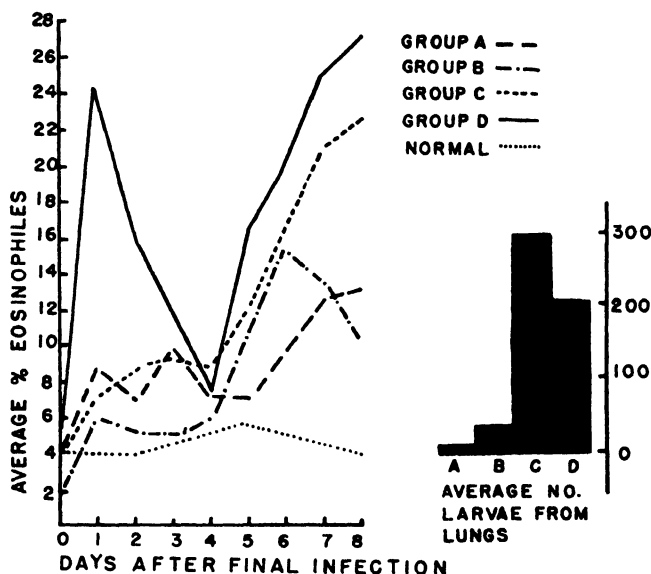
FIG. 16. *Similar to Fig. 13—except guinea pigs in resistant group had received last immunizing infection seven weeks previously.*

The average number of larvae recovered eight days after infection from the lungs of the guinea pigs that had received respectively, three immunizing infections (Group A), one immunizing infection (Group B), no previous infection (Group C), and injections of antigen (Group D), are given in Table III and illustrated in Figs. 13 to 18.
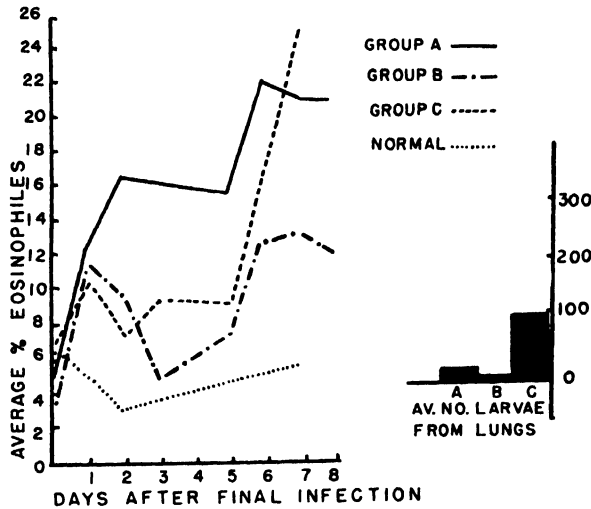
FIG. 17.   *Similar to Fig. 13—except last immunizing infections had been given 10 weeks previously.*
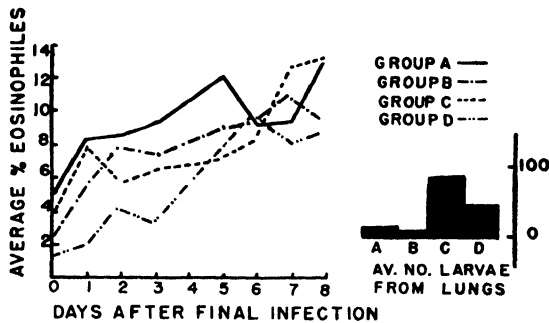
FIG. 18.   *Similar to Fig. 13—except last immunizing infection had been given 15 weeks previously.*

It would appear from the number of larvae recovered from the lungs (Table III), that guinea pigs that received three infections at intervals of seven to nine days (Group A) were more resistant to subsequent infections than those that had received, previously, a single infection (Group B). It is also apparent that guinea pigs were partially resistant to infection with *A. lumbricoides* for at least 15 weeks following a previous infection. (The smaller number of larvae recovered from the pigs in the latter part of the experiment is probably due to fewer living larvae in the eggs fed. Approximately the same number of eggs from the same lot were fed throughout the experiment. It is possible,

TABLE III

AVERAGE NUMBER OF LARVAE RECOVERED, EIGHT DAYS AFTER INFECTION, FROM LUNGS OF
FOUR GUINEA PIGS IN GROUPS A, B, C, AND D, AT INTERVALS FOLLOWING
IMMUNIZING INFECTIONS GIVEN TO GROUPS A AND B AND ANTIGEN
INJECTIONS TO GROUP D

Weeks after last immunizing infection	Average number of larvae recovered			
	Group A, three immunizing infections	Group B, single immunizing infection	Group C, control	Group D, received antigen
1	+	5	507	349
3	2.5	23	280	
5	16	51	105	
7	9	35	298	208
10	25	8	96	
15	16	6	92	48

too, that the age and size of the animals may have had some influence on the result, although an effort was made to minimize such an effect by selecting the larger animals at the beginning of the experiment.) Injections of *Ascaris* antigen gave the pigs relatively insignificant protection against the parasites.

It is apparent from an examination of the results given in Figs. 13 to 18 that *Ascaris* infection produces a marked eosinophilia in guinea pigs. Moreover it reaches higher levels in guinea pigs that have been infected repeatedly at short intervals. It is evident, also, that the eosinophilia is not related directly to the resistance, although such an erroneous conclusion might have been made if the experiment had included only the results shown in Fig. 13. Injections of antigen may produce an eosinophilia but they do not produce a resistance comparable to that which is present following infection. Moreover the eosinophilia may be similar in the animals in different groups but the animals in one group may be much more resistant than those in another (Figs. 14 and 18).

Localization of Resistance

Two groups of guinea pigs, with 16 animals in each, were selected. The guinea pigs in one group were infected twice; those in the second group received a single infection. Two pigs from each group were sacrificed daily for eight days beginning 16 hr. after infection.

The number of larvae recovered from the animals in the two groups (Table IV and Fig. 19) provide additional evidence of the resistance that is produced by infection. It appears from the number of larvae recovered from the livers and lungs, that the body defences, in resistant animals, act against the larvae even before they reach the liver as fewer larvae were recovered from the livers of resistant animals than from those of nonresistant animals. It is evident also that more larvae reached the livers in resistant animals than succeeded in reaching the lungs. This suggests that the body defences are strongly mobilized to act against the larvae before they reach the lungs.

TABLE IV

AVERAGE NUMBER OF LARVAE IN LIVERS AND LUNGS OF TWO RESISTANT AND TWO NONRESISTANT
GUINEA PIGS ON SUCCESSIVE DAYS FOLLOWING INFECTION

Time after infection	Resistant		Nonresistant	
	Liver	Lung	Liver	Lung
Hours				
16	36	0	13	0
Days				
2	30	0	86	0
3	15	1	112	0
4	26	1	84	18
5	24	0	1	46
6	2	2	2	142
7	2	2	1	38
8	0	2	1	112
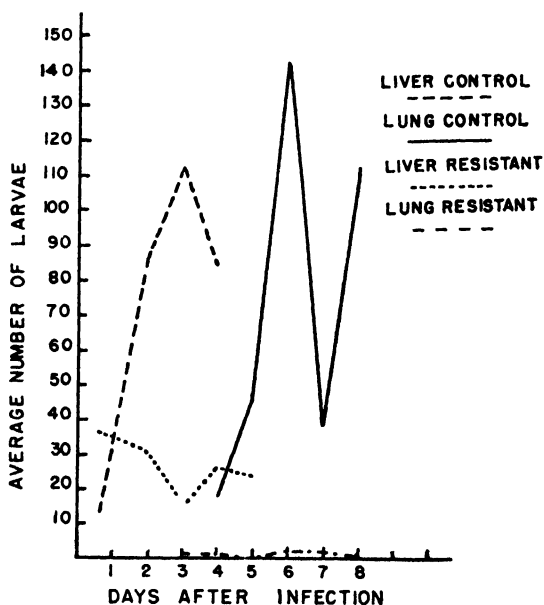
FIG. 19.   Average number of larvae recovered from livers and lungs of two resistant and two nonresistant guinea pigs on successive days following infections.

Lungs of all the guinea pigs that were killed during the first three days following infection appeared normal. The livers in the resistant guinea pigs contained many small lesions similar to those described by Schwartz and Alicata (13) and Oldham and White (10) in hogs, similar lesions were scarcely noticeable in the control guinea pigs during the first three days following infection and were never as numerous as in the livers of guinea pigs from the resistant group.

The measurements of the larvae obtained from the livers and lungs of resistant and nonresistant animals were combined with similar data from a previous experiment (Table I) to construct Fig. 20. Each point shown on
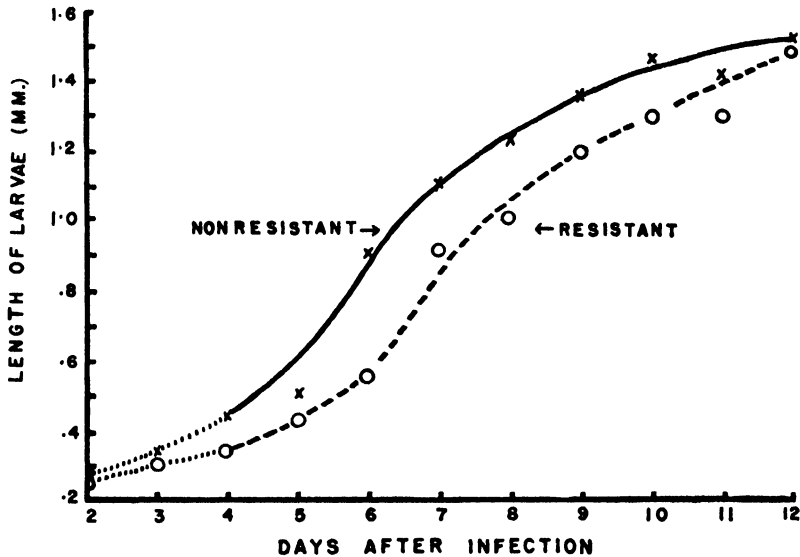
FIG. 20. *Average size of larvae in livers and lungs of resistant and nonresistant guinea pigs on successive days following infection. Measurements made two, three, and four days after infection on larvae recovered from livers, the rest on larvae from the lungs.*

Fig. 20 is based on the average of at least 14, and usually 20 or more, measurements except that for 12 days in the resistant group, which is the average of six measurements. It is apparent from these results that, except on the 12th day following infection, the larvae recovered on any one day were, on the average, smaller in resistant guinea pigs than in those that were not resistant to infection.

Effect of Liver Extract from Resistant Guinea Pigs

Five groups, with three guinea pigs in each, were treated as follows:

Guinea pigs in Groups I and II were given two infections 14 days apart.

Guinea pigs in Group III were infected once, and thus constituted the control group.

Guinea pigs in Group IV were infected once and received 1 cc. intraperitoneal injections of 'normal' liver extract beginning the day prior to infection and continuing for eight days.

Guinea pigs in Group V were infected once and received injections of 'resistant' liver extract at the same time and in the same quantities as that given to the pigs in Group IV.

The pigs in each of the groups were sacrificed by stunning and cutting the jugular veins seven days after infection. The gross appearance of the lungs

from each of the animals is shown in Fig. 6. It will be observed that the lesions were pronounced in the lungs of the pigs that were infected only once and in those that were infected once and received injections of normal liver extract. The lesions were least extensive in the animals that had been infected twice. The lungs of the pigs that received resistant liver extract were more congested than those from pigs that had been infected twice but less congested than those from animals that received normal liver extract. Most larvae were recovered from the lungs that showed the most congestion (Table V).

TABLE V

AVERAGE NUMBER OF LARVAE RECOVERED FROM LUNGS OF GUINEA PIGS SEVEN DAYS AFTER INFECTION

Group	No. pigs in group	Description	Average no. larvae
I	3	Resistant as result of infection two weeks previously	1
II	3	Resistant as result of infection two weeks previously	1
III	3	Controls—single infection	434
IV	3	Received liver extract from normal pigs	146
V	3	Received liver extract from resistant pigs	76

It appears from this experiment that resistant liver extract given intraperitoneally to guinea pigs at the time of infection gave them some protection.

Effect of Serum from Resistant Guinea Pigs

Nine guinea pigs were given 2 to 3 cc. of 'resistant' serum intraperitoneally for five days. Nine pigs were given similar amounts of 'normal' serum in the same way. Each pig was fed several thousand *Ascaris* eggs two days after receiving the first injection of serum. A third group of nine pigs was kept as control and infected at the same time as the pigs receiving sera. The pigs were sacrificed seven days after being infected. The lungs (Fig. 7) of the guinea pigs that received resistant serum were less congested than the others. The number of larvae recovered from the guinea pigs in the different groups is given in Table VI.

The difference between the means of the number of larvae recovered from animals that received resistant serum and from those in the other groups was significant as shown by application of the *t* test. The variability in the number of larvae recovered from the guinea pigs receiving normal serum is a striking, although unexplained feature.

It would appear from these experiments that some passive resistance may be conferred on guinea pigs, at the time of their infections, by intraperitoneal

TABLE VI

NUMBER OF LARVAE RECOVERED FROM GUINEA PIGS THAT RECEIVED 'RESISTANT' SERUM,
'NORMAL' SERUM, AND NO SERUM AT THE TIME OF INFECTION

Resistant serum	Normal serum	Control
17	157	148
4	372	84
3	123	89
5	222	96
1	54	94
49	44	75
5	206	137
14	9	162
12	16	91
Av. 12	Av. 134	Av. 108

injections of sera from guinea pigs that were infected with the parasite three weeks previously.

The average percentage of eosinophiles in the peripheral blood of the guinea pigs receiving resistant serum was higher than in those animals that received injections of normal serum, but the former showed a marked drop on the ·eighth day. It should not be concluded however, that this difference in the eosinophile level is, itself, responsible for the resistance observed.

Discussion

The results of the experiments outlined above confirm those of other investigators who have found that animals that are heavily infected with *Ascaris* have marked symptoms and may even succumb as a result of the infection. The congestion and damage to the lungs were striking pathological features but unlike the congestion resulting from certain types of respiratory infections no elevation of temperature was observed. Blackie (2), from his histopathological studies on *Parascaris equorum*, concluded that two factors, (1) mechanical and (2) toxic, were responsible for the lesions produced. In the lung the mechanical damage overshadowed the toxic effects that were associated, he thought, with the hepatic and renal lesions that were responsible for death. Roberts (12) arrived at rather similar conclusions from his studies on *Ascaris lumbricoides*.

A high eosinophilia was associated with heavy infections and this eosinophilia reached even higher levels when second, third, and fourth infections were superimposed upon. the first before the number of eosinophiles had returned to normal. A marked increase in basophiles, which replace the eosinophiles in foxes, was observed by Kennedy and Law (7) following the administration of *Ascaris* eggs to these animals. The increase appeared to be directly proportional to the number of eggs given. This is of interest because in the present experiments no direct relationship was found between the number of eggs fed and the number of larvae recovered from the tissues.

Injections of *Ascaris* antigen also caused an increase in the number of eosino-philes but the number returned to the normal level more rapidly than was the case following infection.   In a number of infections the eosinophilia developed more rapidly in guinea pigs that had been infected previously.   This fact suggests that the eosinophiles may have an important role in the resistance that was demonstrated quantitatively to exist in guinea pigs that had received multiple infections.   The importance of the eosinophile in this connection is suggested further by the fact that, in a number of animals, the resistance was most marked in those that showed the highest eosinophilia.

The present experiments suggest, however, that the eosinophiles are not solely responsible for the resistance observed, for injections of antigen produced a high eosinophilia, but rendered guinea pigs only slightly resistant to infection. Moreover, evidence was produced to show that a degree of passive resistance could be conferred on guinea pigs by injecting large quantities of serum from resistant animals or extracts from livers of resistant animals.   The lesions observed in the livers together with the number of larvae recovered from the livers and lungs of resistant animals suggests further that substances, or cells, or both, in the liver play an important part in the resistance observed. Some of the cells involved in the liver are probably eosinophiles but other factors are likely concerned also.   Other work on the role of eosinophiles and other factors in resistance in ascariasis is in press (Sprent and Chen (14)).   It may be that the eosinophiles are a result, rather than a cause, of resistance.

The high eosinophilia associated with infection in resistant animals together with the gross appearance of their lungs suggest a possible relation between resistance to *Ascaris* in guinea pigs and sensitivity.   The emphysema that appeared in the lungs of guinea pigs following infection with *Ascaris* and that was manifest sooner in resistant animals than in those nonresistant to infection also supports this view.   It was found that by the time the emphysema appeared in the nonresistant animals, namely 8 to 12 days after infection, that the animals had developed a resistance to the parasite as shown by feeding a second dose of *Ascaris* eggs at this time.   This resistance could be due to antibodies as their production would be expected about one to two weeks after infection.   The decline in resistance that was noticed in animals infected 3 to 15 weeks after their last immunizing infection could be related to a decline in antibodies.   The passive resistance observed as a result of injections of serum from guinea pigs infected three weeks previously could be related also to antibodies.

The resistance appeared to affect the size of the larvae to be found in the tissues on any one day.   The difference in size was apparently a result of a difference in rate of growth rather than a difference in the rate of migration of the larvae through the tissues as it will be observed from Fig. 9 that the maximum number of larvae was found in the lungs of resistant animals about the same time as in nonresistant animals.

Acknowledgments

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References

1. BACHMAN, G. W. and RODRIGUEZ MOLINA, R. Resistance to infestation with *Trichinella spiralis* in hogs. The eosinophilic and precipitin response. Am. J. Hyg. 18 : 266-294. 1933.
2. BLACKIE, W. K. Histological observations on experimental ascariasis. J. Helminthol. 8 : 93-102. 1930.
3. BROWN, H. W. and OTTO, G. F. The differential leukocyte count associated with hookwork infection. Am. J. Diseases Children, 61 : 727-733. 1941.
4. DEBOER, E. Experimentelle untersuchungen über *Ascaris lumbricoides* des Menschen und des Schweinen. Z. Infekt. Hyg. Haustiere, 48 : 248-279. 1935.
5. FALLIS, A. M. Use of the Waring Blendor to separate small parasites from tissues. Can. J. Pub. Health, 34 : 44. 1943.
6. HADWEN, S. Ascariasis in horses. J. Parasitol. 12 : 1-10. 1925.
7. KENNEDY, A. H. and LAW, R. G. The differential blood changes in ascariasis in foxes. Can. J. Research, 12 : 277-285. 1935.
8. KERR, K. B. The cellular response in acquired resistance in guinea pigs to an infection with pig *Ascaris*. Am. J. Hyg. 27 : 28-51. 1938.
9. MORGAN, D. O. Some observations on experimental ascariasis in pigs. J. Helminthol. 9 : 121-128. 1931.
10. OLDHAM, J. N. and WHITE, E. G. Chronic focal interstitial hepatitis in the pig: its experimental production by feeding *Ascaris* eggs. J. Comp. Path. Therap. 54 : 1-15. 1944.
11. RANSOM, B. H. and CRAM, E. B. The course of migration of *Ascaris* larvae. Am. J. Trop. Med. 1 : 129-155. 1921.
12. ROBERTS, F. H. S. The large roundworm of pigs *Ascaris lumbricoides* L. 1758. Bull. 1, Animal Health Station, Yeerongpilly, Queensland, Australia. 1934.
13. SCHWARTZ, B. and ALICATA, J. E. *Ascaris* larvae as a cause of liver and lung lesions in swine. J. Parasitol. 19 : 17-24. 1932.
14. SPRENT, J. F. A. and CHEN, H. H. Immunological studies on mice infected with larvae of *Ascaris lumbricoides*. J. Infectious Diseases. In press.
15. WAGNER, O. Immunisierungsversuche bei experimenteller *Askaris* infektion der Maus. Z. Immunitäts. exptl. Therap. 78 : 372-382. 1933.

ON THE SPECIES OF THE GENUS *METABRONEMA* YORKE AND MAPLESTONE, 1926, PARASITIC IN TROUT AND CHAR[1]

By L. P. E. CHOQUETTE[2]

Abstract

In this study of species of the genus *Metabronema* parasitic in trout and char the author is of the opinion that, on the basis of the characters of the postcloacal papillae and the spicules, the following species should be regarded as synonymous: *Metabronema* (= *Spiroptera*) *salvelini* Fujita, 1920, *Metabronema harwoodi* Chandler, 1931, *Metabronema canadense* Skinker, 1931, and *Metabronema truttae* Baylis, 1935. By virtue of the law of priority, *Metabronema salvelini* Fujita, 1920, must stand as the valid name of the species.

Three species of nematodes ascribed to the genus *Metabronema* Yorke and Maplestone, 1926, have been reported from fish in North America: *Metabronema canadense* described by Skinker (10), *M. harwoodi* described by Chandler (3), and *M. wardlei* described by Smedley (12). The first two species are parasitic in char (*Salvelinus fontinalis*), the third in a species of rockfish (*Scorpaenichthys marmoratus*). The form described by Chandler was first ascribed to the genus *Cystidicola* Fisher, 1798, but afterwards was transferred by Skinker (11) to the genus *Cystidicoloides*. Baylis, in 1933 (1), in his study of the genera *Cystidicola*, *Metabronema*, and *Cystidicoloides* concludes that the last two are synonymous. Therefore, the name of the species described by Chandler became *Metabronema harwoodi*.

Species of *Metabronema* have been reported from char and trout in other parts of the world. In Japan, Fujita in 1920 (Baylis (2)) described *Spiroptera salvelini* from *Salvelinus malma* and, in 1928, reported this species from *Salvelinus kundscha*. Baylis (2) placed this species in the genus *Metabronema*, and, in 1935, Yamaguti (14) amplified its morphological description as *Metabronema* (*Cysidicola*) *salvelini*. In 1928, Fujita (4) reported *Metabronema* (*Cystidicola*) *iwana* from *Salvelinus malma* and, in 1939 (5), three additional species from the char *Salvelinus kundscha*, namely *M. kosugii*, *M. amemasu*, and *M. ishii*. The latter species was described originally under the name of *Metabronema salvelini* but, the name being preoccupied, it was later changed by its author (6) to its present one.

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[2] *Research Assistant and Lecturer in Parasitology.*

In Great Britain, Baylis (2) described *Metabronema truttae* from the brown trout, *Salmo trutta*. Baylis notes the great similarity existing between this species and *Metabronema (Spiroptera) salvelini* and *Metabronema canadense*. He even points out the fact that if all the measurements are taken into account, no clear distinction appears to exist between these forms. This is not the case with the species from the char as described by Fujita (4, 5), all of which possess morphological characters sufficient to distinguish them from *Metabronema salvelini*, *M. canadense*, and *M. truttae*.

During a recent survey of helminths parasitic in the speckled trout, *Salvelinus fontinalis*, in the lakes and rivers of the Laurentide Park, Quebec, a nematode ascribed to the genus *Metabronema* was found commonly in the digestive tract of the host. It was similar to *Metabronema canadense* as described by Skinker (10) from the same host. Since Skinker's description was incomplete, particularly in regard to the nature of the postanal papillae, because of the paucity of the material at her disposal, the opportunity is taken to add to the description of this species and to attempt to clarify its relationship with the others found in char and trout. As stated by Baylis (2) the evidence for the distinctiveness of *M. salvelini*, *M. canadense*, and *M. truttae* is very inconclusive; it rests mainly on morphological points such as the nature of the postcloacal papillae and the character of the spicules. These points were, therefore, given particular study in the specimens found by the writer.

The writer had the opportunity of studying part of Miss Skinker's material but it was, unfortunately, in such a condition as to make very difficult the task of securing a clear picture of the nature of the postanal papillae in the male, and could be studied only in lateral view. This study could only confirm the observations made by Mr. J. T. Lucker (8) of the Zoological Division of the U.S. Bureau of Animal Industry, Washington, D.C., namely, that there were five pairs of postcloacal stalked papillae; that is, one pair additional to the four shown by Skinker in her figure of the right side of the tail of the male. This additional pair is located in close proximity and median to the anteriormost of the postcloacal pair.

· A ventral–dorsal view of the posterior extremity of male specimens, collected by the author, shows that there are five pairs of stalked postcloacal papillae and a sixth pair of very small papillae situated at the posterior extremity of the tail between the fifth pair (Fig. 1); it is doubtful whether they can be seen in lateral view. A sixth pair exists in *Metabronema truttae* as described and illustrated by Baylis (2). No mention of such papillae in *M. salvelini* appears in Fujita's description (Baylis (2)), nor Yamaguti's (14). In the arrangement of the first two pairs of postcloacal papillae there is a similarity between our material and *M. truttae* as described and illustrated by Baylis (2); it also resembles the arrangement described by Yamaguti (14) in respect to *M. salvelini* Fujita.

In his description of *M. truttae*, Baylis (2) states that the longer spicule is the left one while neither Fujita (Baylis (2)) nor Yamaguti (14) state whether the

longer spicule is that of the right or the left side.   In *M. canadense*, according
to Skinker (10), the right spicule is the longer one.   This could not be deter-
mined with certainty in our study of Skinker's material.   However, in the
material at the writer's disposal the longer spicule, as is the case in *M. truttae*
Baylis, is the left one (Fig. 1).   Fujita (Baylis (2)) defines and illustrates
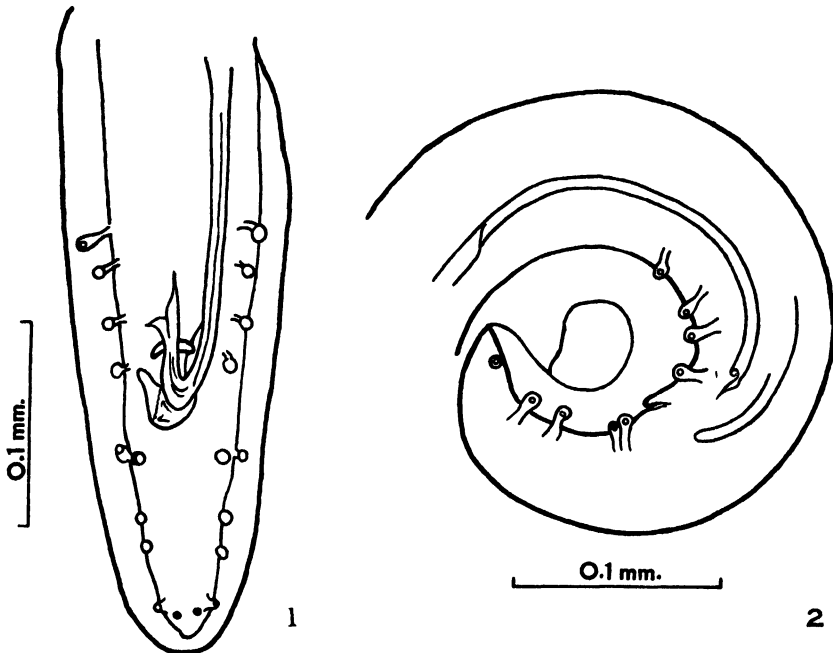
FIG. 1.   *Metabronema canadense, adult male, ventral view.*
FIG. 2.   *Metabronema canadense, adult male, lateral view.*

(4) a pair of transverse processes as the tip of the longer spicule.   This is not
reported by Yamaguti (14), and Baylis was unable to observe whether or not
similar processes are to be found in the form of *Salmo trutta* because he did not
have the opportunity of studying protruded spicules.

Processes on the longer spicules could be seen in ventrodorsal view of the
posterior extremity of another male in mounted specimens (Fig. 1).   However,
the writer is of the opinion that this is not the real aspect of the extremity of
the longer spicule, but that these "transverse processes" are due to distortion
brought about by pressure.   In lateral view it is seen that in its distal extre-
mity the longer spicule is curved into a trough or gutter with the inferior wall
of this gutter prolonged as a sharp point, while the superior one curves inward
(Fig. 2), thus accentuating the groove.   Therefore, when the spicule is pro-
truded and bent on itself, as is often the case, and slight pressure is applied,
so-called "transverse processes" are produced, as shown in Fig. 1.   Skinker
(10), in her figure of the right side of the tail of the male of *M. canadense*,
shows partly the arrangement existing at the distal extremity of the longer
spicule.   This aspect of the spicule was observed also in her type material.

The aspect of the distal end of the spicule in lateral view is quite similar to that illustrated by Johnston and Mawson (7) in their study of *Ascarophis cooperi* from *Platycephalus bassensis*.

Lyster (9) reported the presence of *Metabronema (Cystidicoloides) harwoodi* in speckled trout from other parts of this Province. However, examination of Lyster's material shows it to be identical with *M. canadense*. This species was found also in material from a species of char (probably the Arctic char, *Salvelinus arcturus* Günther), from the coast of Labrador, and deposited at this Institute.

Van Cleave and Mueller (13) report the presence of *Metabronema* (= *Cystidicoloides*) *harwoodi* Chandler in *Salmo fario* from Oneida Lake. These authors, after study of Chandler's type material, conclude that this species is identical with *M. canadense* Skinker, this species being merely a smaller variety. In their redescription of *M. harwoodi*, these authors report the presence of five pairs of postanal papillae and discuss the shape of the longer spicule at its distal extremity. The writer had the opportunity of studying specimens kindly loaned by Prof. Chandler, as well as the type material of *M. harwoodi*, deposited in the helminthological collection of the United States National Museum. In both cases the specimens were found to exhibit the character used as criterion in this study of species of the genus *Metabronema* in char and trout, that is, the arrangement of the postanal papillae and the shape of the left spicule.

Conclusions

As the result of this study, the writer believes that, on the basis of the characters of the postcloacal papillae and the spicules the following species should be regarded as synonymous: *Metabronema* (= *Spiroptera*) *salvelini* Fujita, 1920, *Metabronema harwoodi* Chandler, 1931, *Metabronema canadense* Skinker, 1931, and *Metabronema truttae* Baylis, 1935. By virtue of the law of priority, *Metabronema salvelini* Fujita, 1920 must stand as the valid name of the species.

Acknowledgments

References

1. BAYLIS, H. A.   Three helminthological notes.   Ann. Mag. Natural History, 14 : 115-121.   1934.
2. BAYLIS, H. A.   Four new species of nematodes.   Ann. Mag. Natural History, 16 : 370-382.   1935.
3. CHANDLER, A. C.   New genera and species of nematode worms.   Proc. U.S. Natl. Museum, 78 : 1-11.   1931.
4. FUJITA, T.   Further notes on nematodes from fishes of Lake Biwa.   (Japanese with English summary).   Dobutsugaku Zasshi, 40 : 309-314.   1928.

5. FUJITA, T. On the nematoda-parasites of the Pacific salmon. J. Fac. Agr. Hokkaido Imp. Univ. 42 : 239-266. 1939.

6. FUJITA, T. New names for *Metabronema salvelini* Fujita and *Cystidicola minuta* Fujita. J. Parasitol. 27 : 542. 1941.

7. JOHNSTON, T. H. and MAWSON, P. M. Some parasitic nematodes from South Australian marine fish. Trans. Roy. Soc. South Australia, 69 : 114-117. 1945.

8. LUCKER, J. T. Personal communication. April, 1948.

9. LYSTER, L. L. Parasites of freshwater fish. II. Parasitism of speckled and lake trout and the fish found associated with them in Lake Commandant, Que. Can. J. Research, D, 18 : 66-78. 1940.

10. SKINKER, M. S. Three new parasitic nematode worms. Proc. U.S. Natl. Museum, 79 : 1-9. 1931.

11. SKINKER, M. S. A redescription of *Cystidicola stigmatura* (Leidy), a nematode parasitic in the swim bladder of salmonoid fishes, and a description of a new nematode genus. Trans. Am. Micro. Soc. 50 : 372-379. 1931.

12. SMEDLEY, E. M. Some parasitic nematodes from Canadian fishes. J. Helminthol. 12 : 205-220. 1934.

13. VAN CLEAVE, H. J. and MUELLER, J. Parasites of Oneida Lake fishes. Part 3. A biological and ecological survey of the worm parasites. Roosevelt Wild Life Ann. 3 : 1-334. 1934.

14. YAMAGUTI, S. Studies on the helminth fauna of Japan. Part 9. Nematodes of fishes, I. Japanese J. Zool. 6 : 338-386. 1935.

A PRELIMINARY ACCOUNT OF THE BITING FLIES AT CHURCHILL, MANITOBA[1]

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Abstract

The occurrence is recorded at Churchill, Manitoba, of 5 genera and 11 species of Culicidae, 2 genera and 12 species of Simuliidae (3 and possibly 4 of which may be new to science), and 2 genera and 10 species of Tabanidae. Data are presented on their habitats, life histories, habits, species association and succession, and relative abundance and distribution. Observations on the relationships of these insects to other organisms are recorded, including notes on their status as pests and their influence on human activities in the locality. Evidence is presented that female mosquitoes feed on the nectar of flowers and are efficient pollinators of northern orchids. A brief general picture of the ecology of the locality is given; also details of weather conditions during the period of the survey, and some microclimate data. Illustrations from photographs showing typical habitats of many of the species dealt with are included.

Introduction

The observations on which this paper is based were made during the course of a biting fly survey and experimental control program carried out at Churchill, Manitoba, during the spring and summer of 1947 as a joint project of the Division of Entomology, on behalf of the Canadian Defence Research Board, and the U.S. Bureau of Entomology and Plant Quarantine, on behalf of the Surgeon General, Department of the U.S. Army*. The results of the control investigations and repellent studies against the various species of biting flies are to be published elsewhere.

Churchill is situated at approximately latitude 59° N. and longitude 94° W., on the western shore of Hudson Bay, at the mouth of the Churchill River. It lies inside the subarctic area, about 170 miles from its northern boundary. Within a radius of a few miles is to be found a great variety of terrain, including spruce–larch forest, muskeg, tundra, tundra meadow, birch–willow scrub, low gravel and granite ridges, tidal flats, and sand dunes. It is a zone of transition from forest to tundra and supports a varied insect fauna including species of biting flies typical of the forest and the plain and of the subarctic and the arctic.

The northern fringe of the vast subarctic forest extends to within a short distance of Churchill. In the Churchill region it is rather open and swampy and consists chiefly of spruce and larch (the tallest trees probably not exceeding 30 ft.) and dwarf shrublike willow and birch. The forest floor is for the most part soft and spongy underfoot, extremely tiring to walk on, and in many places treacherous to the unwary. It frequently has a variegated appearance owing to the different shades of gray, green, and brown of the deep mat of lichens, mosses, and grasses that cover it. The trunks and branches of the trees commonly bear patches and tufts of lichens, which increase the rather unkempt and melancholy appearance of the somber woods.

The tundra and tundra meadow in the area extends from the scrubby and scattered tree growth along the uneven margins of the forest to the shore of Hudson Bay. The predominant vegetation consists of lichens, mosses, and coarse grasses. Flowering plants abound, their successive blooming adding a touch of color and beauty to the bleak landscape. Where soil conditions are favorable, dwarf spruce grows in small clumps or singly. On exposed ridges overlooking Hudson Bay the branches of these stunted trees grow only on the south side of the slender trunks, giving them a forlorn, windswept appearance. In protected situations, such as along the borders of streams and on the swampy ground between the forest and the Churchill River, dwarf birch and willow grow, in places forming dense thickets.

Large numbers of birds nest and raise their young in the Churchill region and the marshes and swamps often resound with their calls. Common among them were ducks, geese, gulls, terns, sandpipers, plovers, curlews, snow buntings, and ptarmigan. Among them, too, was that familiar habitué of suburban gardens, the American robin. Mammals were much less in evidence. A few caribou were seen near the Churchill River; also porcupine were seen in the forest and a number of larch trees stripped of bark by their feeding. Local trappers stated that the woods south of Churchill provide good winter hunting of moose and caribou and trapping of mink and beaver. Burrows and trails of small rodents were numerous in the area, but their numbers were apparently at a low ebb in 1947, for the animals themselves were rarely seen and, in spite of several attempts to trap them, only three arctic mice (lemming) were captured. From these, two males and six females of the flea *Megabothris groenlandicus* Wahlgren were taken (det. G. P. Holland). Schools of white whales entered the mouth of the Churchill River after the ice went out (June 20) and numbers of these were harpooned and shot for their meat and oil by local Indians and trappers. Of fish, pike, grayling, and suckers abound in the larger streams and apparently serve as a major part of the diet of sled dogs during their enforced summer idleness. Specimens of two small species of fish taken in the streams were sent to the Royal Ontario Museum of Zoology, Toronto, Ont., and identified by W. B. Scott, of the Division of Fishes, as the capelin, *Mallotus villosus*, and the nine-spined stickleback, *Pungitius pungitius*. No reptiles were seen, but frogs were common in the marshes

and swamps, and specimens collected were identified by Clyde L. Patch, of the National Museum, Ottawa, as the northern wood frog, *Rana sylvatica cantabrigensis*.

Drainage is generally poor in the Churchill region because much of the terrain is low-lying and the ground is permanently frozen a short distance beneath the surface. As a result, innumerable shallow pools form on the tundra (Figs. 1 and 2) and in the forest during the spring thaw and serve as breeding places for the countless hordes of mosquitoes that appear on the wing in late June and July. What drainage there is occurs largely through numerous streams that flow into the Churchill River and the Hudson Bay. From these streams and the rapids of the river, vast numbers of blackflies emerge and, with the mosquitoes and the bloodsucking tabanids variously called bulldog, moose, and deer flies that develop in the forest, plague man and beast throughout the short summer.

The Weather

An analysis of daily weather summaries obtained from the Department of Transport meteorological officer at Churchill for the 87-day period (May 16 to Aug. 10) covered by this investigation, shows that the average temperature was 47° F., the averages for May (part), June, July, and August (part) being 24.5, 44.8, 58.3, and 54.3° F., respectively. The range of temperature was 73 Fahrenheit degrees, from a low of 9° F. on May 17, to a high of 84° F. on June 20 and July 12. The relative humidity was high throughout the period but particularly so before the thaw. The average figure for the whole period was 85.5%; that for May (part) 91.5%; and that for the remaining period, separately, 84%. The relative humidity figures had a range of 57% from a low of 43 on June 18, to 100% on several days; daily averages ranged from 63 on June 19, to 100%. No definite trends appear in the figures for barometric pressure.

Wind speed showed a slight general decrease from May to June and July, followed by an increase early in August. The average wind speed for the whole period was 12.6 m.p.h.; averages for each of the months separately were 14.3, 11.8, 11.9, and 14.7 m.p.h. respectively. The range of wind speed was from calm on May 18, July 13, July 16, and Aug. 3, to 45 m.p.h. on May 24, and 36 m.p.h. on Aug. 6. Apart from a scarcity of east and southeast winds, there was no marked trend in the frequency with which the wind blew from each direction, except that west and northwest winds were somewhat the more common.

The total precipitation during the period was 5.18 in., water equivalent, of which 0.08 in. was in the form of snow. This precipitation was distributed between the four months as follows: 0.18, 0.91, 0.95, and 2.90 in., the maximum rainfall on any one day being 1.63 in. on Aug. 5. There was measurable precipitation on 36 of the 87 days: on 7 of the 16 days in May, 10 days in June, 12 days in July, and 7 of the 10 days in August.

PLATE I
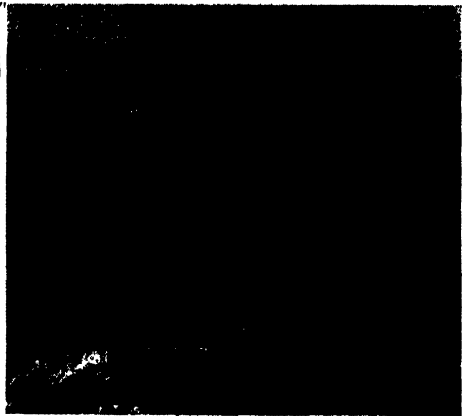
FIG. 1. *Mosquito infested pools on tundra in early June.* FIG. 2. *Snowpools on tundra meadow, breeding place of A. nigripes and A. punctor.* FIG. 3. *Beach Bay area east of Churchill River, breeding place of several species of Aedes.* FIG. 4. *Pools near Beech Bay, breeding place of A. nearcticus and A. campestris.* FIG. 5. *Forest snow pools in which newly hatched larvae of A. communis and A. punctor appeared on June 9.* FIG. 6 *Pollinium of the northern orchid, Habenaria obtusata, attached to the compound eye of A. nigripes.*

PLATE II

FIG. 7. *Railway drainage channel, breeding place of S. venustum.* FIG. 8. *Larvae of S. venustum photographed on stones under water.* FIG. 9. *Lesions on neck caused by bites of S. venustum.* FIG. 10. *Small stream or rill where larvae and pupae of E. latipes were found.* FIG. 11. *Mideastern Creek near source on tundra, breeding place of Eusimulium and Simulium species.* FIG. 12. *Tabanids (H. affinis) in flight around a stationary vehicle.*

Observations of cloud conditions during the hours of daylight may be summarized as follows: 53% overcast, 40% partly cloudy, 7% clear.

The ice went out of the Churchill River on June 20, the expected date for this occurrence being that of the spring tides closest to June 15. The season at this stage was nearly a week later than normal. A comparison of average temperatures for June and July, 1947, with similar averages obtained over a number of years supports the impression received from residents that by the end of July the season was normally advanced.

The Mosquitoes (Culicidae)

THE SPECIES PRESENT

Eight species of *Aedes* and two species of *Culiseta* have been identified among the specimens collected or reared in the Churchill area during 1947. No other genus of the subfamily Culicinae was represented, but the predaceous larvae of three genera of nonbiting mosquitoes of the subfamily Chaoborinae, namely, *Eucorethra*, *Mochlonyx*, and *Chaoborus*, were fairly common in the culicine breeding pools. Unfortunately, none were reared to the adult stage, but a male and female collected from a mating swarm at 4 p.m. on July 24 belonged to the species *Chaoborus nyblaei* Zett.

The two species of *Culiseta* represented are *C. alaskaensis* Ludl., and *C. impatiens* Wlk. They are not abundant in the area, only small numbers of overwintered females being seen or captured during the month of June. No males or aquatic stages of the species were found.

The species of *Aedes* definitely determined include *A. nigripes* Zett., *A. punctor* Kby., *A. nearcticus* Dyar, and *A. communis* Deg., occurring probably in that order of abundance; *A. campestris* D. & K. and *A. excrucians* Wlk., less abundant, but numerous and widespread; *A. flavescens* Müll., and *A. cinereus* Mgn., apparently relatively few and localized in occurrence. From the examination of numerous females in the temporary field laboratory at Churchill, it was believed that several other species of *Aedes* were present in the region, but it was not possible to substantiate this in the absence of males, or the definite association of reared females with the last larval molt.

Dr. Alan Stone, of the Division of Insect Identification, of the U.S. Bureau of Entomology and Plant Quarantine, Washington, D.C., who kindly examined the doubtful specimens, stated, "I do not feel confident of determining any of your undetermined female *Aedes*. There is just too much similarity and at the same time too much variation within species to permit it, I think." Later, he further commented (*in litt.* Jan. 12, 1948) "As for the females, the more I look at them the less sure I am that they can be determined with any degree of accuracy. I tried to sort them out, but I was so uncertain that I

finally gave up. I think it quite probable that you have no other dark-legged species than these four (*nigripes*, *nearcticus*, *punctor*, and *communis*), with the possible exception of one specimen that looked very much like *pullatus*. I am quite certain that neither *aurifer* nor *intrudens* were present; *cataphylla* may be present, but I very much doubt it. Since *pionips* does not appear to be separable from *communis* in either the female or the male genitalia, it is possible that this species is included in those which I have determined as *communis*. It would be necessary to see larvae to settle this point." Incidentally, it is worth noting that there is no clear indication of the difficulty or impossibility of determining these and certain other black-legged northern species in Matheson's key (8) for the identification of North American *Aedes* females.

SEASONAL DEVELOPMENT

All the species of *Aedes* found in the Churchill area overwinter in the egg stage. Larvae began to hatch in small numbers in snowpools in sheltered places on the tundra at the beginning of June, when snow and ice were still prevalent in the area. By the middle of June, when the snow was largely gone from the tundra, the larval population of several abundant species was nearing its peak in pools in the open and among the sparse tree growth along the margins of the forest. These species were *A. nigripes*, *A. punctor*, and *A. nearcticus*. Immature larvae of *A. excrucians* had also begun to appear. In the forest the first larvae of *A. communis* were found hatching in favored snowpools on June 9, when nearly two-thirds of the forest floor was still covered with fast-melting snow.

Pupation of *A. nigripes* began on June 16, and *A. punctor* on June 17, the first adults emerging on June 20 and June 21, respectively. Emergence of *A. nearcticus* started on June 22, and that of *A. communis* in the forest on June 24. Periodical collections indicate that these four dark-legged species are the dominant ones in the Churchill region.

Towards the end of June emergence was general. During the early part of July pupae were still common, but larvae were becoming increasingly hard to find. By July 3, tremendous numbers of mosquitoes were on the wing and they continued to increase until a peak of abundance was reached during the second week of July. The pest species were augmented by adults of *A. campestris* on July 5, *A. excrucians* on July 9, *A. cinereus* on July 13, and *A. flavescens* on July 20. By the third week of July, however, a definite falling off in abundance and aggressiveness of the general mosquito population was apparent. This is reflected in the daily average number of bites per minute on exposed untreated forearms (elbow to wrist) and legs (knee to ankle) recorded during 100 check tests carried out at several points in the area, July 6 to 17, in connection with field studies of repellents (Fig. 13).

After the third week in July the decline in mosquito abundance was progressively more marked, until in early August, when observations ceased, their numbers had reached comparatively small proportions.
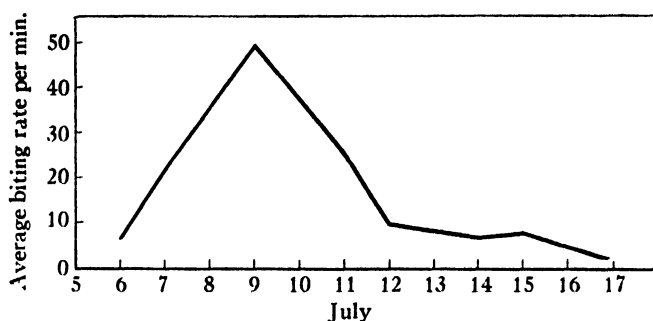
FIG. 13. *Graph showing average number of mosquito bites per minute on forearms and legs in 100 check tests, Churchill, July 6 to 17, 1947.*

NOTES ON THE SPECIES

Aedes nigripes Zetterstedt

This rather large, hairy, dark-colored species is the most abundant mosquito in the Churchill region beyond the forest.

Larvae began to hatch in shallow, grassy, snow water pools on tundra meadow (Figs. 1 and 2) early in June. Small numbers of first, and fewer early second, instar larvae were found on June 4. The water in these pools was 52° to 64° F., but was probably lower at night, some of the pools having a thin sheet of ice on them in the morning, before the sun was high.

By June 11, when the snow had largely disappeared from the open tundra except for occasional dwindling drifts, newly hatched larvae were still appearing, but second instar larvae predominated and many had molted into the third stage. The general infestation in the innumerable pools across the marshy tundra and among the scattered trees along the margins of the swampy forest ranged from sparse to moderate: in some there were none; in others one to several larvae per square foot; in still others several to many per dip.

Fourth instar (last stage) larvae of *A. nigripes* began to appear in sheltered pools on June 13, associated with lesser numbers of *A. punctor*. The first pupae were found on June 16, in shallow pools on rough meadow supporting a sparse scattered growth of stunted spruce and larch. The temperature of the water was 73° F. Immature larvae were still plentiful in some pools.

A typical breeding area of *A. nigripes* is near Beech Bay, east of the Churchill River, consisting of several square miles of shallow pools among marsh grasses and dwarf willow and birch (Fig. 3). In addition to *A. nigripes*, larvae of *A. nearcticus*, *A. excrucians*, and *A. campestris* developed in this area. When examined on June 18, the numerous larvae ranged from first to fourth instar, probably second and third predominating. The smallest larvae may have been *A. campestris*.

June 20 was one of the warmest days of the summer, the shade temperature reaching 84° F. Pupae were becoming increasingly common in the breeding areas. By 9 a.m. several males of *A. nigripes* emerged in the laboratory and emergence had undoubtedly started in the field. The next day (June 21) parties working at various points in the area reported that mosquitoes were on the wing and beginning to be troublesome. Specimens collected were *A. nigripes*, *A. nearcticus*, and *A. punctor*. On June 26, the pools in the Beech Bay area were still infested with larvae and pupae. The heaviest infestation appeared to be in the transition zone between grass and dwarf willow and birch, consisting almost entirely of fourth instar larvae, but some pupae. Higher up in the shrub zone the infestation was at least 50% pupae. Larvae collected were 86% *A. nigripes*, the remainder being *A. excrucians* and *A. campestris*. Emergence of adults of *A. nigripes* was increasingly general throughout the area during the last 10 days of June and was probably largely completed in the first week of July, the last recorded emergence being on July 4.

By July 3, mosquitoes *(A. nigripes, A. nearcticus,* and *A. punctor)* were present in tremendous numbers and continued to increase for some days thereafter. *Aedes nigripes* is a strong flier and probably a migratory species, although its flight range has not been determined. This statement also applies to *A. nearcticus.* On the night of July 2–3 there was a strong south-southeast wind and for the first time the insects were numerous in the military camp and below the ridge overlooking Hudson Bay, and many persons donned headnets and there was a sudden call for repellents. Outside the camp, in the sparse woods and on the tundra they occurred in enormous numbers and with a sharpened blood lust. Even on the granite and gravel ridge exposed to a strong cool wind they beat at one in clouds, with darting, dancing flight, getting into mouths, ears, and nostrils and up trouser legs and into pockets, biting at every opportunity, wherever the skin was unprotected by clothing or repellent.

At 1.15 p.m. on July 3, several mating swarms were seen over the tundra between a narrow belt of scrubby spruce and the camp buildings. They occurred in small darting, dancing clouds around the perimeter of a small, shallow lake, the swarms extending about three to nine feet above the ground. A midget net swept several times through two of these swarms caught a couple of dozen males and several females, all *A. nigripes.*

On July 6 two observers walked several miles east of the camp along the rocky Hudson Bay coast line, skirting the granite ridge to the marshy tundra meadow beyond, to examine blackfly streams. In spite of a fresh northeast breeze and bright sunshine, mosquitoes were abundant everywhere, although less numerous on the sandy beach by the sea away from cover of any sort. Among large rocks and boulders, however, they were as troublesome as inland on the tundra. They even sheltered in collections of seaweed left high by the retreating tide. They were persistent in attack, flying against the wind.

A mixture of equal parts of dimethyl phthalate and ethyl hexanediol (Rutgers 612) applied to the skin had to be renewed at less than hourly intervals. The insects alighted in large numbers on hats and clothing. Khaki woollen shirts gave only partial protection and it was found necessary to apply repellent freely to the cloth by hand. The mosquitoes readily found untreated places and collected there in clusters, biting freely where the cloth was in contact with the skin. Specimens collected indicated *A. nigripes* to be the dominant species in these open places but *A. nearcticus* was present, and probably also *punctor*.

On July 9, a visit was made by assault boat to several points along the west bank of the Churchill River from opposite Churchill to Mosquito Point. The weather was misty, humid, and moderately warm, the wind light, and mosquitoes were on the wing in enormous numbers. The terrain here consists of marshy ground with patches of dwarf willow, gently sloping upwards to a granite ridge. Many of the mosquitoes were largely denuded of scales on the mesonotum, but a careful examination of the more perfect specimens indicated that *A. nigripes* was most numerous, with *A. nearcticus* and *A. punctor* also present. They continually beat at one, sometimes sounding like rain against the parka jackets. Blackflies (*Simulium vittatum* Zett.) were discerned among the swarming mosquitoes. Repellent 6–2–2 smeared liberally on all exposed areas of skin generally prevented biting, but 'the applications had to be renewed every half hour or so. Elsewhere in the area biting rates of up to 120 per minute, with an average of 50, on exposed, untreated forearms, or legs from knee to ankle, were recorded by investigators making field tests of repellents.

A. nigripes had probably reached or passed its peak this day. After the middle of July there was a marked decline in the abundance and aggressiveness of this and other black-legged species of *Aedes* in the area, but they persisted in diminishing numbers into August.

There are few definite records of the distribution of *A. nigripes*, but, with *A. nearcticus*, it is probably widespread throughout arctic Canada. It was first collected, as larvae, from a pond at Bernard Harbour, N.W.T. (north of the Arctic Circle) on June 28, 1915, by Fritz Johansen and described as *Aedes* n. sp. by Dyar (2). In 1925, as recorded by Twinn (14), J. D. Soper collected males, females, and larvae east of Nettilling Lake, Baffin Island (near the Arctic Circle), the larvae being taken on June 28, and the adults on June 30, July 9, 17, and 22. According to Twinn: "During his sojourn in Baffin Land, Mr. Soper· states that mosquitoes were not very troublesome, and it was never necessary to wear a veil as a protection against their bites. He found larvae common in shallow pools in the latter part of June, the adults making their appearance at the end of June, and becoming most numerous in late July and early August, apparently reaching the peak of their abundance on August 7." In 1947, T. N. Freeman collected females of *A. nigripes* at Baker Lake, N.W.T. in the latter part of July and a male on Aug. 1.

Aedes nearcticus Dyar

This species is closely allied to *A. nigripes* and hard to distinguish from it in the adult form.   For this reason it is rather difficult to evaluate its status as a pest in the Churchill area on the basis of the 1947 data, especially as males were reared from larvae collected in only three areas, all treeless, and including the marshy tundra just east of the Churchill River at Beech Bay (Fig. 4), tundra meadow pools, and grassy pools on the granite ridge between Churchill and the camp.   These reared males, eight in all, emerged June 22 to 26, indicating that the life history of the species is similar to that of *A. nigripes*.

Numerous females collected and identified as *A. nearcticus* by means of Gjullin's key (3) indicate the species to be an abundant one in the region, although this was not revealed in a study of collections of larvae taken from various breeding places in the area.   Like *A. nigripes*, this species is believed to be a strongly migratory one, and the infestation may have originated largely from breeding places outside the surveyed area.   The distribution of *A. nearcticus* is not well known.   It is probably widespread in the Canadian arctic, and it occurs at high elevations farther south.

Dyar (2) described *A. nearcticus* from specimens reared by Fritz Johansen at Bernard Harbour, N.W.T. in July 1915.   He also identified as apparently the same species, females collected by Johansen at Young Point, Cockburn Point, and Cape Bathurst, N.W.T. and Herschel Island, Y.T.

Hearle (4) reported localized breeding of *A. nearcticus* at high altitudes in the Rocky Mountains in Alberta.   Localities mentioned include Lake Louise in 1921, Simpson's Summit (about 7000 ft.) in 1922, and the Cascade Mt. amphitheater in 1924 and 1925.   In the Cascade Mt. area in 1925 Hearle noted that pupation was general by June 18 and the adults began emerging by June 22, which is similar to the seasonal development observed at Churchill (at less than 75 ft. above sea level) in 1947.

Aedes punctor Kirby

Like *A. communis*, this species is difficult to separate in the female form from the other black-legged *Aedes* (except *A. cinereus*) in this region.   It is an abundant pest in the Churchill area, fiercely attacking man and beast in the forest with *A. communis*, and over the tundra with *A. nigripes* and *A. nearcticus*.

The larvae of *A. punctor* were most commonly found associated with those of *A. nigripes* and less frequently with *A. communis* and *A. excrucians* in pools and marshy areas sparsely grown with scrubby spruce and larch; with *A. nigripes* on treeless open tundra meadow beyond but near the tree line, and with *A. communis* in the swampy woods (Fig. 5).

The earliest *A. punctor* larvae began hatching in favored grass-bottomed snow water pools beyond the forest in the first week in June, and in the forest on June 9.   First stage larvae collected from a tundra meadow pool on June 6 and from a forest pool on June 9 and reared in the laboratory produced both sexes of adults on June 29.

In the field, mature larvae were numerous by June 14 in grassy pools in the open, and among or near sparse tree growth and in open places in the forest. The earliest pupae appeared on June 17, and the first adults (identified by the male genitalia) were on the wing on June 21. Mature larvae could be found up to the end of June, and emergence of adults from specimens taken to the laboratory continued up to July 7, by which date the general mosquito population was reaching its peak.

Males of *A. punctor* were collected swarming a few feet above the Hudson Bay Railway tracks, in the forest near Warkworth Creek, after sunset (10.30 p.m.) on July 13. They were also collected swarming over the tundra near woods east of Churchill, at 4 a.m. (about 10 min. after sunrise) on July 25.

The species is widely distributed in Canada from Prince Edward Island to British Columbia. The most northerly record for Canada so far obtained is Baker Lake, N.W.T., where eight males were collected by T. N. Freeman, July 14 and 23 and Aug. 2 and 13, 1947 (det. G. E. Shewell). According to Hearle (6) it does not fly far from its breeding places.

Aedes communis DeGeer

This black-legged forest species is very similar in appearance to *A. punctor* Kby., from which it is probably distinguishable with certainty only in the larval form or by the male genitalia. *A. communis* and *A. punctor* appear to be the dominant mosquitoes throughout the vast swampy coniferous forest south of Churchill.

First stage larvae were found in shallow snow water pools in the open forest (Fig. 5) several miles south of the tree line, on June 9. On this day nearly two-thirds of the forest floor was still snow-covered (to a depth of 2 to 3 ft. in places), lakes were frozen, and most of the numerous pools and ponds formed by melting snow had snow or ice in them or at the margins. It was apparent that hatching of the overwintered eggs was not general as larvae could be found only in occasional favored pools in sunlit places sheltered from the wind. The temperature of one small pool heavily infested with newly hatched larvae was 48° F.

Three days later, on June 12, after a spell of comparatively warm weather another visit 12 miles south in the forest was made by snowmobile down the tractor trail to Warkworth Creek. By this time more than two-thirds of the forest floor was free from snow, the area was much wetter, and the rough trail was flooded with several inches of water in many places. At several points examinations were made of water bodies on or near the trail. Where coarse grasses were prevalent mosquito larvae in the first and second instars were few to very abundant, several scores being picked up in a single dip. The temperature of the infested pools was generally 50° F. or more. In colder waters the larvae had not yet appeared; nor were any found where the bottom consisted largely of sphagnum and other mosses.

Other activities prevented a further visit to this area until June 24, a cold, wet day, when some of the grassy pools contained innumerable pupae, and larvae and pupae were also found in moss-bottomed pools uninfested on previous visits.

Emergence of adults from material collected in those forest pools commenced in the laboratory the same day (June 24), 31 males and 15 females of *A. communis* emerging between that date and June 29. Other larvae of this species taken from pools in the swampy forest just south of Churchill, and from patches of scrubby spruce–larch woods east and west of the military camp gave rise to 14 males between July 4 and 7.

Unfortunately, it is difficult to identify with certainty the females taken in nature, but the prevalence and abundance of the larvae of *A. communis* indicate that this species formed an important part of the immense numbers of mosquitoes that were an almost intolerable pest to man and beast in and near the forest about Churchill in late June and July. Although it is a great pest in the forest, biting freely at almost any time of the day when its haunts are invaded, but especially after sunset, it is unlikely that *A. communis* flies far from the shelter of woods, or is a pest on the tundra at points distant from the tree line.

Wesenberg-Lund (16) studied the biology of this species in Denmark. He states:

"In the vast forests of North-Seeland I have observed how the swarms in May are restricted to the very ponds in which they are hatched; but later on, especially in the middle of June, the swarms are fused together and, everywhere in the forest, horses as well as man are attacked. As an exclusive forest species it hardly ever goes out of the forest; according to my experience, the attack is always worst in the biggest and darkest part; here in the deep shade the attack on a sunny day at noon is just as severe as in the evening."

A. communis occurs in forested regions across Canada.

Aedes campestris Dyar and Knab

Compared with the abundant, rather drab, black-legged mosquitoes in the Churchill area, this is an attractively marked species, predominantly yellow and brown in color, with wings evenly mottled with dark and pale scales.

It is a rather late appearing species, larvae being first collected on June 26 in shallow, open pools in the marshy meadows in the Beech Bay area, east of the Churchill River (Fig. 4). From these several males emerged in the laboratory on July 5, and a female on July 6. On July 8, a warm sultry day, southward from Churchill to Warkworth Creek, along the railroad track, females of *A. campestris* were observed and specimens collected among the innumerable mosquitoes attempting to bite.

This species was a conspicuous one in open places, especially along the railway track in the vicinity of the camp site at Warkworth Creek, July 13 to 15, in sparse, swampy coniferous forest. It is believed that the source of these mosquitoes was the marshy tundra meadows a short distance to the west, between the river and the forest. The females of *campestris* attack at all times of the day from early morning until well after sunset. The times of day noted between July 13 and July 26 when collections were made of specimens, biting or on the wing, included 4.30 a.m., 11 a.m., 4 p.m., 7 p.m., 9 p.m., and 10 p.m. Specimens with the appearance of having recently emerged were noted as numerous and troublesome in Churchill military camp during the latter part of July, especially towards evening and after sundown, often invading the living quarters to bite. The insects were still common in the open country surrounding the camp in the early part of August when observations ceased.

Matheson (8) notes that *A. campestris* has been "recorded from Michigan west through the northern plains states to Utah, north to Alaska, and east to Hudson Bay." Rees (11) records that, in Utah, the species has been found to have a flight range of 10 miles from its breeding places.

Aedes excrucians Walker

This is a rather large mosquito with uniformly dark scaled wings and the tarsal segments ringed with white, broadly so on the hind legs.

It is a moderately common species at Churchill. The larvae were found in shallow grassy pools in sparsely wooded areas, in the open swampy forest and on the open tundra meadow, appearing somewhat later than the dominant black-legged species. They were found, usually in small numbers, in various locations associated with larvae of *A. nigripes*, *A. nearcticus*, *A. campestris*, *A. punctor*, and *A. communis*.

The larvae were first identified in the third instar on June 14. Mature larvae were collected on June 21, and specimens could still be found up to July 5. Adults were not taken on the wing until July 9, but became increasingly common after that date, persisting in moderate numbers into August. *A. excrucians* females are vigorous biters and may be active day and night, but especially in the evening and early morning. Recorded times of captures at Churchill were 1 a.m., 2. a.m., 6 a.m., 11 a.m., 3 p.m., 4 p.m., 6 p.m., and 7 p.m. According to Hearle (6) they do not fly far.

The species has a wide distribution in Canada, south of the tree line.

Aedes cinereus Meigen

A. cinereus is a small, dark mosquito, the smallest in the Churchill fauna. The larvae were not found, but the species is known to favor shallow forest pools. Females were collected in numbers July 13 to 25, chiefly in the swampy coniferous forest south of Churchill, but also on the tundra east of Churchill, near woods. They were found most numerous near Warkworth Creek at a temporary camp site in the woods, and were recorded biting in or near forest

cover from 9 a.m. to 8 p.m. Those taken in mid-July were in excellent condition as if newly emerged. The adults are reported to stay close to their breeding places. The species is widely distributed in Canada.

Aedes flavescens Müller

A large, yellowish species, with white banded tarsi, *Aedes flavescens* is a late appearing mosquito, individually conspicuous, but of comparatively minor importance at Churchill.

The first females were taken in the lakes and tundra meadow area west of the camp on July 20, about a month after the first appearance of *A. nigripes.* The next day they were observed in fairly large numbers in the open country bordering the Churchill River, together with *A. campestris* and other species They occurred in moderate numbers throughout the remainder of July and until observations ceased in early August, specimens being collected almost daily and always in the open, in and about the camp area and westward to the river. Most of the specimens were females but three males were taken in the Beech Bay area on July 21 and Aug. 2.

This species occurs widely in northern parts of the Nearctic and Palaearctic regions. Its biology has been studied in Canada by Hearle (5) and in Denmark by Wesenberg-Lund (16). The former noted that its main range in North America is in the Great Plains and that it is the second commonest species in the Canadian prairies where it breeds in large semipermanent, moderately deep pools containing much vegetation. In Denmark, Wesenberg-Lund indicated its habitat to be open meadows bordering on lakes and seashores. Both authors reported that although it readily attacks man it prefers larger animals, and when numerous is a serious pest of livestock. It has only one generation annually. The females, which may live for several weeks, are active by day, but especially at dusk. Hearle found that blood meals are essential to the development of the ovaries. The species is believed not to fly far from its breeding places.

Genus Culiseta Felt

Two species of this genus were found to occur sparsely in the Churchill area, namely, *C. alaskaensis* Ludlow and *C. impatiens* Walker. They appear to be too few and too timid in attack to be of importance as pests in this region.

Culiseta alaskaensis Ludlow

This is the largest mosquito in the region and is easily recognized by its size, its spotted wings, and the broad basal white bands on the tarsi.

Females of this species were first seen in the early evening (6.30 to 7 p.m.) on June 5 (a sunny day) flying in and about the officers' quarters, and two were captured. This was about two weeks before the earliest *Aedes* adults appeared. Occasional females were seen, or collected attempting to bite, in the swampy forest south of Churchill between June 9 and June 27, always

during periods of sunshine and, on two occasions (June 9 and June 12), when the air temperature was at or below 50° F., snow was still prevalent in the woods, and ice and snow persisted in forest pools and ponds (Fig. 5). Undoubtedly these insects had hibernated in sheltered situations through the long winter. An indication of the sort of places where *Culiseta* females hibernate is given in Peter Fidler's Journal for Apr. 4, 1792 (as quoted by Dr. Douglas Leechman, National Museum, Ottawa). The entry, which reads as follows, was made near Fort Fitzgerald, northwest of Lake Athabaska, just south of the northern boundary of Alberta:

> "Put up about ½ mile above the head of the rapids amongst a deal of old Large Poplars we made a good fire & soon after we were all very much surprised to see numbers of Muskettoes flying about altho the Snow was more than 10 Inches deep on the ground every where on examination we found that betwixt the Bark of the Poplars & the tree, of the old Dry wood there was a large open space which was full of muskettoes that have been in that situation all winter in some places they was in large cakes of 2 Inches thick the heat of the fire had invigorated them so as to be able to fly about in the manner before mentioned".

Little is known of the life history of *C. alaskaensis*. No adults were seen after the end of June and no aquatic stages were found. The eggs are reported to be deposited in rafts on the surface of water. An unsuccessful attempt to obtain eggs for rearing was made on June 17 by placing several captured females in a large cage containing a tray of water and split soaked raisins. They survived only two or three days without ovipositing. One of them that was induced to take a blood meal at noon on June 18 fed to repletion in two and one-half minutes.

According to Matheson (8) the previously recorded distribution of *C. alaskaensis* in North America was from Alaska to Colorado, the species occurring in the higher mountain ranges in the southern part of its range.

Culiseta impatiens Walker

This is a medium sized species, with black tarsi and less distinct spots on the wings than *C. alaskaensis*. Apparently it has similar habits. Two females were collected in bright sunshine in open spruce–larch woods just south of Churchill, on June 17, one while attacking a dog. It is reported to occur throughout the northern part of North America.

Mosquito Activity Around the Clock

Two observers made hourly collections of biting flies during a 24-hr. period on July 24–25 at two sites, one on tundra meadow and the other in nearby woods at a point seven miles east of Churchill Camp and about a mile from the Hudson Bay shore. The bulk of the mosquitoes collected were dark-legged species mostly too denuded for accurate determination, but probably including *A. nigripes*, *A. nearcticus*, and *A. punctor*. Small numbers of *A. campestris*, *A. excrucians*, and *A. cinereus* were also present.

The dark-legged species were on the wing throughout the 24-hr. period, but were most numerous between sunset and sunrise (10.55 p.m. and 3.50 a.m.) especially in the woods. During this period there was little or no wind and the lowest temperature recorded was 54° F. Males of *A. punctor* were observed swarming shortly after sunrise. *A. excrucians*, too, was collected at dark as well as in daylight. *A. campestris* and *A. cinereus* were collected during the hours before 9 p.m. and after sunrise.

MOSQUITOES AS POLLINATORS

On July 9, on the west bank of the Churchill River, some specimens in the attacking swarms of females of *A. nigripes* were noticed bearing one, and occasionally two, bright yellow stalked bodies attached by an adhesive disc to the ventral margin of the compound eyes (Fig. 6). Later these bodies were found adhering to the eyes of females of several other species of mosquitoes in the Churchill area, including *A. excrucians*, *A. punctor*, and *A. cinereus*. Of female specimens taken at hourly intervals on a 24-hr. collecting trip, on July 24–25, the percentage bearing them ranged from 2 to 33%, the average for the total collections being 6%.

At first these bodies were presumed to be a form of fungus, although they interfered not at all with the blood lust of the insects bearing them. Specimens were sent for identification to specialists in Canada and the United States and caused them considerable puzzlement. Eventually some of the specimens reached Prof. E. B. Mains at the University of Michigan, and Prof. T. Petch, King's Lynn, England, both of whom indicated them to be pollinia of a species of orchid*. The latter reported (in part) ". . . yours are the typical structure of an orchid pollinium—an adhesive disc, a stalk of varying length, and pollen masses at the apex."

The matter was then brought to the attention of Mr. A. E. Porsild, Chief Botanist, National Museum of Canada, who stated that the pollinia were from the northern orchid, *Habenaria obtusata*. He had first observed them on mosquitoes when he was camped on the north shore of Bear Lake, in July, 1928. He further noted that "*Habenaria obtusata* is the most common of the northern orchids and grows in open forests from Newfoundland to Alaska. On Bear Lake and elsewhere I have noted that, during its flowering period, as many as 5 per cent of mosquitoes on my tent carried one or two pollinia on their heads. Several species of orchids are equipped with pollinia that, upon the slightest touch, spring forward and by their sticky, disc-like base attach themselves to the head of an insect."

Mr. Porsild pointed out that this phenomenon had already been recorded by Raup (10). The latter observed the pollinia on the heads of mosquitoes in the Athabaska – Great Slave Lake region, and stated: "It is possible that the great abundance of the orchids (*H. obtusata*) is due to an efficient pollinization carried on by the myriads of mosquitoes which inhabit the woods."

McClure (9) has recorded three species of *Habenaria* at Churchill, namely, *H. obtusata* and *II. hyperborea* in forested or bush areas, and *Habenaria* sp. in the bush and on high and mixed tundra.

The Blackflies (Simuliidae)

THE SPECIES PRESENT

In the Churchill area blackflies breed in great numbers in the small streams that flow into the Churchill River and into Hudson Bay. They also develop in great abundance in the rapids of Warkworth Creek, south of Churchill, and the Churchill River itself. The difficult nature of the terrain, limited transport facilities, and the preoccupation of personnel with other projects permitted only an incomplete study of this group of insects.

Some years prior to the 1947 survey, one of the authors (Twinn) examined and identified collections of blackflies taken at Churchill in 1934, by A. M. Heydweiller, and in 1936 by H. E. McClure.

Heydweiller's collection, all females except where otherwise indicated, included six species that are listed as follows, together with a statement of the numbers of specimens and the period during which they were collected:

> *Simulium venustum* Say, 121, July 11 to Aug. 30
> *Simulium vittatum* Zett., 34 (plus four males) July 8 to Aug. 14
> *Simulium ottawaense* Twinn, 26, July 10 to Aug. 15
> *Simulium arcticum* Mall. 4, July 30
> *Simulium perissum* D. & S., 4, July 30 and Aug. 11
> *Eusimulium baffinense palens* Twinn, 2, July 30

McClure's collection was made up of many hundreds of female specimens, mostly *S. venustum* and *S. vittatum*, but also including four specimens each of *S. arcticum* (July 11 to 23) and *E. subexcisum* Edw. (July 11).

Thus these two collections were comprised of seven species. Among the specimens collected or reared during 1947, eight species are represented, five of them being additional to those collected by Heydweiller and McClure, making a total of 12 species found in the Churchill region. It is probable that other species will be discovered when further studies are made. The eight species taken in 1947 are as follows:

> *Simulium venustum* Say
> *Simulium vittatum* Zett.
> *Simulium* sp.
> *Eusimulium aureum* Fries
> *Eusimulium latipes* Mgn.
> *Eusimulium* species A
> *Eusimulium* species B
> *Eusimulium* species C

NOTES ON THE SPECIES TAKEN IN 1947

Simulium venustum Say

This species is the most abundant of the blackflies in the Churchill area and the most important pest of man. The immature stages were found in all bodies of running water examined wherever blackflies were developing, including drainage ditches (Fig. 7), rills (Fig. 10), larger streams (Fig. 11), and rivers.

The first pupae were discovered on July 3, in a small stream in the camp area and adults began emerging on July 5. Observations on several other streams indicated that emergence was general in the area on this and subsequent days. Females were first captured attacking humans on July 8, by which date mosquitoes were reaching their peak. By mid-July, the numbers of blackflies of this species were tremendous, especially in wooded areas, and the streams contained heavy infestations of eggs, larvae (Fig. 8), and pupae, a condition that persisted until early August, when observations ceased.

In the latitude of Ottawa, *S. venustum* passes the winter in the larval stage (15) but in the Churchill region it is believed to overwinter in the egg stage, except possibly in the Churchill River, which continues to flow all winter beneath the ice. The smaller streams were frozen solid in the winter of 1946–7 and thawed out during the first week of June. At this time small numbers of dead pupae and cocoons of *S. venustum* from the previous season were found attached to stones and vegetation in several of the streams, but no living pupae or larvae were seen, and young larvae did not begin to appear until after the middle of June, two to three weeks before the first adults of this species were on the wing.

By the latter part of July, observations and reports indicated that *S. venustum* had become a more troublesome pest than mosquitoes, their numbers increasing as the latter diminished. Collections at hourly intervals around the clock, on July 24–25, at a point about seven miles east of the camp, revealed that about 90% of the blackflies taken were this species. They were active throughout the day, but none were caught in the hours of darkness between 11 p.m. and 3 a.m.

Many persons experienced trouble from the flies crawling into their clothing and biting various parts of their bodies. One woman in the camp stated that they got into her young son's hair and bit his scalp, resulting in an infection requiring medical attention. Frequently the bites are not felt when inflicted, but later, hard lesions form that may become very itchy (Fig. 9). Sometimes the insects fly about one in clouds and, even when not biting, cause annoyance by getting into eyes, mouth, and ears.

There appear to be at least two generations of *S. venustum* at Churchill and probably three or a partial third, the generations overlapping, so that after the first adults emerge all stages may be found together throughout the short summer season. Apparently development continues until the

streams freeze up. According to an official of the Churchill grain elevator, adult blackflies are present in the area in varying numbers "until the snow flies".

S. venustum is widely distributed and one of the most important pest species of blackflies in Canada (15).

Simulium vittatum Zetterstedt

Female specimens of *S. vittatum* were collected in the open and in the woods at a location east of the Churchill River, south of Churchill, on June 21. These were the first blackflies on the wing, and presumably developed from larvae that had overwintered in the rapids beneath the ice in the Churchill River. The ice went out of the river on June 20. Pupal skins of this species were found on submerged rocks in the rapids close to the shore line, at Mosquito Point, on July 9. The pupae were not found in any of the numerous smaller streams examined in the region.

On July 9, also, great numbers of *S. vittatum* were intermingled with the hordes of mosquitoes on the west bank of the Churchill River. Several sweeps of a midget net caught 264 mosquitoes and 11 males and 52 females of *S. vittatum*. There was no evidence that this species attacked humans.

Of 265 blackflies taken in hourly collections during a 24-hr. period on July 24–25, only two or 0.7% were *S. vittatum*. The locality was about 10 miles east of the Churchill River. If, as observations indicate, the breeding of this species is confined to the river in this area, the distance might explain their scarcity at that point. *S. vittatum* is widespread throughout Canada and occurs in the arctic, females having been collected at Lake Harbour, Baffin Island, by W. J. Brown in August, 1935 (15).

Simulium sp.

The material referred to as *Simulium* sp. consisted of two pupal cases in cocoons collected on a stone taken from the Churchill River rapids at Mosquito Point on July 9. The cocoons are boot-shaped, and the respiratory tufts of the pupae are short and comprised of 16 filaments. The material may represent an undescribed form.

Eusimulium latipes Meigen

This species was found in a shallow, stony, rather swift, little stream or rill (Fig. 10) that has its source in a pool in the camp area and flows through open woods and marshy meadows towards the Churchill River.

Numerous well-grown larvae and small numbers of pupae were found on stones and grasses in the stream on July 3. These were the first blackfly pupae seen in the area. The water temperature was 59° F. By the next day the temperature had risen to 63° F. and pupae were much more numerous. The majority of them were *Eusimulium latipes* Mgn., the remainder being *Simulium venustum* Say. Adults of the latter species began emerging on July 5, and *E. latipes* on July 7. Several collections were made and by

July 12 numerous adults of both species had emerged, the earlier emergences being predominantly males. By this date the pupae in the stream were mostly *venustum*, *latipes* being only sparsely represented.

By July 20, no immature blackfly stages could be found in the stream, nor were any discovered during subsequent examinations on July 28 and Aug. 6.

This species, which is widespread in Europe, was first recorded in North America near Hull, Que., by Twinn (15). It apparently has only one generation a year.

Eusimulium aureum Fries

Six females were collected on July 24, July 25, and Aug. 2. No pupae were found. This species is widely distributed in North America.

Eusimulium species A

Both sexes of this species were obtained from pupae taken from three streams designated in our notes as F.S.B.-E., F.S.B.-M.E., and F.S.B.-W.T., the first being the Eastern Creek, a shallow stream flowing into Hudson Bay· five miles east of the camp, the second a small stream (Fig. 11) about midway between Eastern Creek and the camp (dubbed Mideastern Creek), and the third a shallow little stream originating in Lake Isabelle and flowing past the water tower in the camp area towards the Churchill River.

Emergence of adults was actively proceeding from Mideastern Creek on July 6, and may have begun a day or two earlier, as on this date empty pupal skins greatly outnumbered pupae. Newly emerged specimens were crawling up the vegetation in large numbers and many of the flies were also on the wing, but careful observation of them was impeded by the presence of clouds of aggressive mosquitoes. Emergence of this species from the other two streams was in progress about a week later.

This appears to be a single generation species that overwinters in the egg stage, as evidenced by observations on stream F.S.B.-W.T. at approximately weekly intervals from the time the stream began to flow shortly after the thaw in early June, until early August. These data on *Eusimulium* sp. A, breeding in this stream, follow:

June 15 and June 22, no larvae; June 29, large numbers of larvae newly hatched to two-thirds grown; July 4, larvae abundant, many maturing, no pupae; July 12, many pupae adults emerging and mating freely, even when imprisoned in a glass container; July 20, July 28, and Aug. 6, no pupae of this species found, the infestation being entirely *S. venustum*.

Females of *Eusimulium* species A were collected on the wing on July 24 and 25. This is apparently an undescribed species. Dr. Alan Stone, of the U.S. Bureau of Entomology and Plant Quarantine, who has retained the material for further study, states that it is close to *E. minus* D. & S. The pupa has short, bushy respiratory tufts, each consisting of 25 to 30 filaments, arranged mostly in pairs on short stalks arising from a common base. The cocoon is of indefinite shape.

Eusimulium species B

About 10 miles south of Churchill the two main arms of the Warkworth Creek flow northwestward beneath the railway to the Churchill River. The southern arm, about 60 to 100 ft. wide in the vicinity of the railway, is referred to locally as the Goose River.

On July 8, the Goose River was 4 to 5 ft. deep and flowing 3 to 4 ft. per second. The water temperature was 62° F. Blackfly larvae were extremely numerous and pupae and empty pupal cases abundant on the vegetation and stones in the rapids. The majority of the larvae and many of these pupae were *Simulium venustum* Say, but most of the pupae (in collections taken that day) had respiratory tufts each of eight filaments and, from these, emergence of both sexes of a species of *Eusimulium* was general. The species was not recognized and was designated *Eusimulium* sp. B. Emergence was apparently completed by July 14, only the wall-pocket-shaped cocoons and empty pupal cases of this species being found in addition to the abundant immature stages of *S. venustum.*

Pupae and pupal cases of *Eusimulium* sp. B were taken on stones in the Churchill River rapids at Mosquito Point, on July 9, and one pupa was found in collections from Eastern Creek on July 12.

The material has been submitted to Dr. Alan Stone who stated (*in litt.* Jan. 12, 1948): "I do not recognize this and should think that it is an undescribed species. . . . I am holding the specimens for further study."

Eusimulium species C

This species is represented by one female, which emerged by July 12 from a pupa collected from Mideastern Creek (see under *Eusimulium* sp. A), on July 6. The respiratory tufts of this pupa each consist of numerous filaments (30 to 40) branching out on short stalks from the dorsal surface of a stout elongate main trunk. The pinned specimen, with the pupal case preserved in euparal on a piece of celluloid, has been passed to Dr. Alan Stone.

The Tabanids (Tabanidae)

THE SPECIES PRESENT

Only a relatively casual survey of this group was possible. The material obtained consists of specimens collected during the course of other work, and is comprised entirely of adult females, no males or immature stages having been taken.

The specimens represent six species of the genus *Hybomitra*, locally called bulldog or moose flies, and four species of *Chrysops*, commonly referred to as deer flies. These species, arranged within the genera in order of abundance are: *Hybomitra affinis* Kby., *H. septentrionalis* Loew, *H. metabola* McD., *H. zonalis*

Kby., *H. hearlei* Philip, and *H. gracilipalpis* Hine, the latter represented by a single female; *Chrysops carbonaria* Wlk., *C. furcata* Wlk., *C. nigripes* Zett., and *C. frigida* O.S.*

NOTES ON THE SPECIES

The succession and relative abundance of these species as indicated by the material available are shown in Fig. 14. The first specimens taken were *H. metabola*, collected on July 5. Three independent reports were received

FIG. 14. *Succession and relative abundance of tabanid species at Churchill in 1947.*

of men being bitten prior to this (July 3) by flies that, from their description, could have been tabanids. The first specimens of *H. affinis* were taken on July 6. This rapidly became the dominant species, and remained so until the middle of August (when observations were terminated), although it was in close competition with *H. septentrionalis* from the third week of July onwards.

The earliest species of *Chrysops* to appear was *C. carbonaria*, which was first taken on July 12. This was the most abundant species of the genus until

* *Determinations were made or confirmed by Mr. G. E. Shewell, Division of Entomology, Ottawa.*

towards the end of July, when it gradually gave place to a predominance of *C. furcata*, which, with *C. frigida* and *C. nigripes*, made its first appearance a few days after *C. carbonaria*.

H. zonalis with its striking yellow and black abdomen was remarkable in that it put in an early appearance on July 10, but was not taken thereafter. All the specimens of this species were collected in the camp area.

With the exception of *H. affinis*, no species of either genus was found in appreciable numbers more than a few hundred yards from woodland; this was especially true of the *Chrysops* species, which were seldom taken outside of the woods. The rarer species of *Chrysops* were collected only in woodland some distance from open country.

The abundance of certain species leads to speculation as to the larval food material. Hine (7) successfully reared a number of species of *Hybomitra* on small crustacea and on earthworms, both living and dead. Stone (12) was able to repeat this but found mosquito larvae to be the best food for the young tabanid larvae. It would seem possible, therefore, that the enormous numbers of mosquito larvae stranded from drying pools may form a material element in the diet of one or more species of *Hybomitra*. Cameron (1) states that *Chrysops* larvae will not feed on living animal material.

The adults of several species of *Hybomitra*, *H. affinis* conspicuous among them, are attracted in large numbers to vehicles, including trains as well as the tracked vehicles used in the Churchill area. Numbers of flies would collect around a vehicle (Fig. 12) while an observer standing a few yards away might pass practically unmolested. Stone (12) suggests that they are attracted by the movement, but adds that they will also collect around a standing automobile. This fact was also observed, but in every instance it was around a standing vehicle that had been in motion and was warm in consequence. Warmth appears to be an attractive factor, while movement stimulates pursuit. Certainly it is of no use attempting to run or even to drive away from tabanids over the terrain around Churchill. Specimens that appeared to be *H. affinis* were observed to lose ground only slowly when in pursuit of a train travelling at approximately 30 m.p.h. On the morning of July 12 and a few days subsequent to this, several observers reported a caribou calf apparently separated from the herd, careering madly around the Churchill locality pursued by a small cloud of tabanids. The animal appeared in very poor condition, presumably as a result of the attentions of the flies. Chained dogs, however, were not seen to be attacked by large numbers of tabanids, possibly on account of their inability to run away.

Activity, and especially biting activity, of tabanids is far more influenced by weather conditions than is that of mosquitoes. In cool, damp, overcast, and windy weather few, if any, tabanids were seen on the wing. The weather factors of greatest importance seem to be temperature and sunlight; high relative humidity appears to be associated with absence of biting only secondarily, by virtue of its association with low temperature. Biting was rare at

temperatures below 55° F., and, even above this temperature, would not normally occur unless there had previously been an appreciable period of sunshine on the same day. The well-established preference of Tabanidae for biting through wet skin (13) was confirmed by the observations of personnel working in blackfly streams. During 24-hr. observations on biting fly activity carried out on July 24–25, tabanids were no longer on the wing about a half an hour after sunset. The temperature at that time fell below 65° F. and the light value below 500 lumens per square foot. Activity was not resumed until two hours after sunrise, when the temperature was 55° F. and the light value about 2000 lumens per square foot. *H. affinis* was the most persistent species in each instance, but possibly only apparently so, on account of its greater abundance. The relative freedom of the shore of Hudson Bay from tabanids was more noticeable than its freedom from either mosquitoes or blackflies. Tabanids gained access to buildings in considerable numbers, but caused annoyance there more by their efforts to get out again than by any attempts to bite. In fact, there was no instance recorded of a tabanid biting indoors.

Considerable personal variation in liability to being bitten was noticed. The species of *Chrysops* appear to bite man more freely than do those of *Hybomitra*, but none appears to be a serious biter of man when he is protected by suitable clothing and a good standard repellent such as dimethyl phthalate. All species, however, contribute materially to the psychological menace of the biting fly swarm (mosquitoes, blackflies, and tabanids) that collects about man in this region during the fly season, the *Chrysops* species by virtue of their rather silent approach and furtive alighting and the *Hybomitra* species on account of their swift and noisy flight, and the momentum of their impact on the face and person.

Further survey work on the northern species of tabanids is required to determine what other species occur. No reasonable approach to the problem of controlling the numbers of any one species can be made until the habitats and food materials of the immature stages are more exactly known.

References

1. CAMERON, A. E.   Bionomics of the Tabanidae of the Canadian Prairie.   Bull. Entomol. Research, 17 : 1-42.   1926.
2. DYAR, H. G.   The mosquitoes collected by the Canadian Arctic Expedition, 1913-18. Rept. Can. Arctic Expedition III, Pt. C, 31-33.   1919.
3. GJULLIN, C. M.   A key to the *Aedes* females of America north of Mexico.   Proc. Entomol. Soc. Wash. 48 : 215-236.   1946.
4. HEARLE, E.   Notes on the occurrence of *Aedes* (*Ochlerotatus*) *nearcticus* Dyar in the Rocky Mountains Park, Alberta.   Can. Entomologist, 59 : 61-63.   1927.
5. HEARLE, E.   The life history of *Aedes flavescens* Müller.   Trans. Roy. Soc. Can. Ser 3, Sec. V, 23 : 85-101.   1929.
6. HEARLE, E.   Notes on the more important mosquitoes of Western Canada.   Proc. 19th Ann. Meeting New Jersey Mosquito Exterm. Assoc. 7-14.   1932.
7. HINE, J. S.   Habits and life histories of some flies of the family Tabanidae.   U.S.D.A. Bur. Entomol. Tech. Ser. 12.   1906.
8. MATHESON, R.   Handbook of the mosquitoes of North America.   2nd ed.   Comstock Publishing Co. Inc., Ithaca.   1944.

9. McCLURE, H. E.   Invertebrate ecology of Churchill.   Ecol. Monographs, 13.   1943.

10. RAUP, H. M.   The pollinization of *Habenaria obtusata*.   Rhodora, 32 : 88-89.   1930.

11. REES, D. M.   The mosquitoes of Utah.   Bull. Univ. Utah, 33 (7).   1943.

12. STONE, A.   Bionomics of Tabanidae.   Ann. Entomol. Soc. Am. 23 : 261-304.   1930.

13. STRICKLAND, E. H.   Unpublished communication.

14. TWINN, C. R.   Mosquitoes from Baffin Land.   Can. Entomologist, 59 : 47-49.   1927.

15. TWINN, C. R.   The blackflies of Eastern Canada (Simuliidae, Diptera).   Can. J. Research, D, 14 : 97-150.   1936.

16. WESENBERG-LUND, C.   Contributions to the biology of the Danish Culicidae.   D. Kgl. Danske Vidensk. Selskab. Skrifter, Naturvidenskab. math. Afdel., 8 Raekke. 7 : 1-120. 1921.

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